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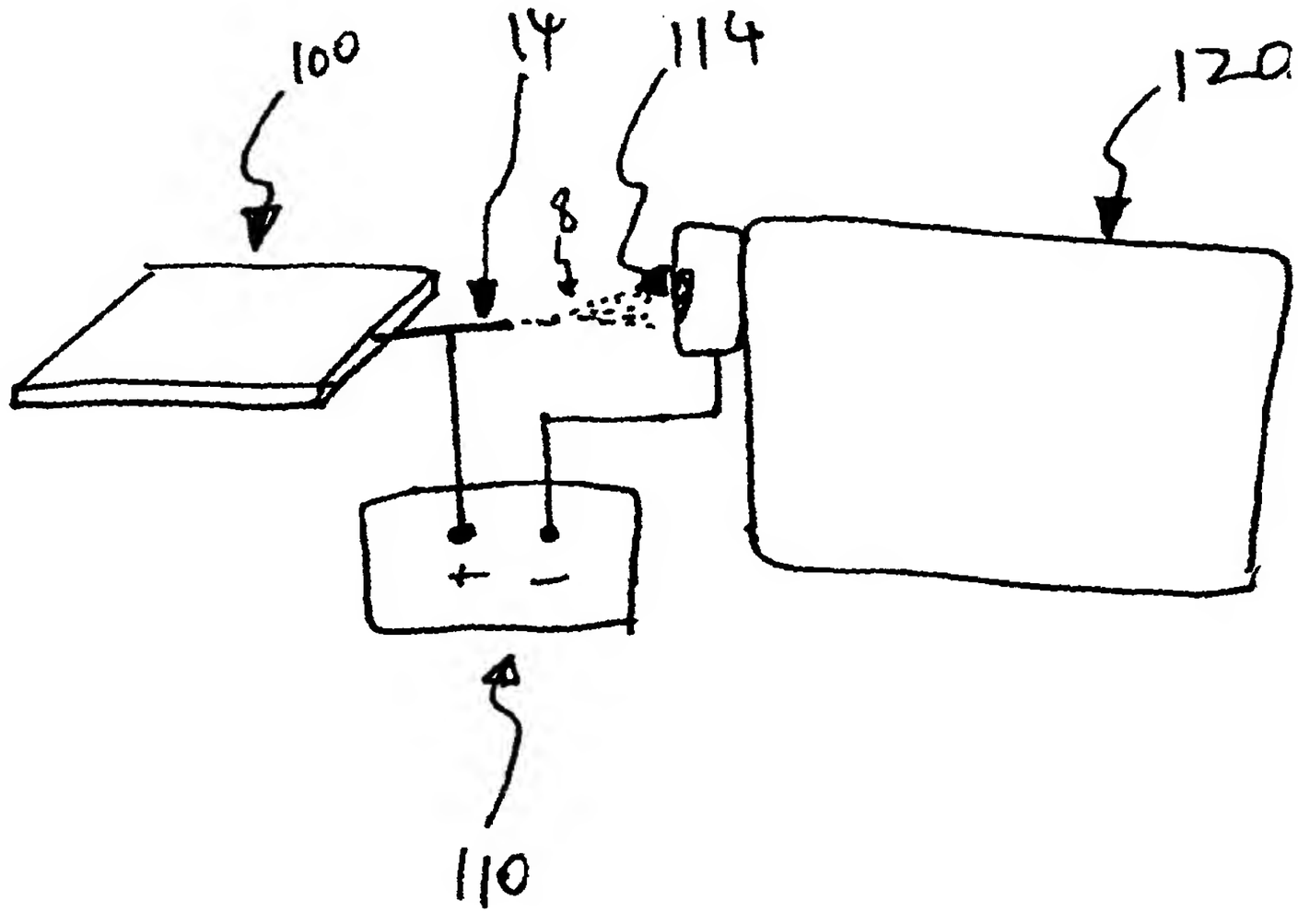
(54) Title: MICROFLUIDIC DEVICE BASED SAMPLE INJECTION SYSTEM FOR ANALYTICAL DEVICES



(57) Abstract: This invention provides a microfluidic sample injection apparatus for injecting a fluid sample into an analytical device and a method for using the same. The microfluidic sample injection apparatus comprises a microfluidic device and an integrated sample injection capillary which is in fluid communication with a fluid flow channel of the microfluidic device.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Microfluidic Device Based Sample Injection System for Analytical Devices

FIELD OF THE INVENTION

[01] This invention relates to a microfluidic sample injection apparatus for injecting a fluid sample into an analytical device and a method for using the same.

BACKGROUND OF THE INVENTION

[02] Recently, microfluidic devices capable of conducting chemical reactions and assays on a single microchip have been developed. However, the method of detection has been mostly limited to laser-induced fluorescence (LIF) because of its simplicity and sensitivity. One of the limitations of LIF is that it requires the analyte of interest to be fluorescent. Since most compounds are not natural fluorophores, LIF is not an ideal detection method. Thus, in order to use LIF detection method, a derivatization step is often required to make compounds of interest amenable to LIF detection.

[03] Mass spectrometry (MS) is currently being investigated as an alternative detection method for microfluidic devices. In this regard, electrospray ionization mass spectrometry (ESI-MS) is particularly suited due to the similarity in flow rates generated by the microchip (i.e., microfluidic device) with those required for ESI-MS. ESI-MS is a powerful tool that has been broadly applied to the structural analysis of biological molecules. In particular, it provides a facile means to interface liquid chromatographic (LC) systems and mass spectrometry (MS), creating a system that integrates separation with structural analysis and molecular identification. The development of LC-MS has revolutionized analytical chemistry and biochemistry.

[04] In the post-genomic era, attention has turned from DNA sequencing to the more complex problem of analyzing how this genetic information directs cell function. The analysis of protein structure and function is one of the keys to this question. In particular, analysis methods currently under development are typically focused on identifying unknown proteins whose presence can be correlated with a function, disease state or reaction to potential drug candidates.

[05] Mass spectrometry is a highly sensitive tool for the analysis of proteins. It enables the masses of fragment ions of proteins or peptides to be determined with high

accuracy and with high sensitivity. High mass accuracy enables an accurate and specific sequencing of peptides. In combination with progress in genomic sequencing and bioinformatics, this enables the identification and characterization of unknown components of cells. In tandem with multidimensional gel electrophoresis methods, it provides a means to identify the complement of the proteins expressed by a cell under a defined set of conditions. This totality of expressed proteins is defined as the proteome.

[06] Mass spectrometry is also developing from this simple "mining tool" for providing protein sequence information into more deeply integrated areas, such as functional characterization of biologically important genes, functional proteomics, quantitative mapping of cellular proteins and deciphering protein interaction networks. In addition to sequencing, mass spectrometry is currently the only tool available that can readily detect post-translational modifications (changes to protein structure after synthesis), such as phosphorylation and dephosphorylation and the actions of proteases that each plays critical roles in the control of cellular activity.

[07] Another important MS application is the identification of molecules participating in the formation of macromolecular complexes. The study of molecular interactions is a rapidly developing field. The analysis of protein expression in cells (also known as proteomics) is therefore important in target identification and validation, and in ADME/PK (absorption-distribution-metabolism-excretion/pharmacokinetic) studies. However, such proteomic studies, in which proteins are identified by analysis of enzymatically produced peptide fragments, are expensive and labor-intensive. Technical difficulties exist in both sample separation and sample delivery systems for using ESI-MS in analysis of proteins, primarily because the samples that can be isolated from traditional gel-based electrophoresis are in very limited amounts. This makes them difficult to analyze in a traditional ESI-MS configuration.

[08] To overcome some of the problems created by small sample sizes, interfaces capable of delivering low nanoliter per minute volumes of sample (so-called 'nanospray') to MS have been developed. These extend the time over which a very small amount of sample (e.g., 1 μL or less) can be delivered to the mass spectrometer, providing improved signal/noise ratios and thus sensitivity. However, Nano-ESI-MS is labor-intensive and slow (in current designs, sample loading and set-up of the electrospray capillary are both manual processes). In addition, it cannot be readily adapted to on-line capillary separation methods such as liquid chromatography or

capillary electrophoresis. For these reasons, nanospray is most often used as a "static" or off-line method in which samples are analyzed one-at-a-time, representing a serious bottleneck in applications that requires high throughput. Software that integrates the variety of analytical methods required to perform high throughput analysis using these systems is already available, thus design of a robust multi-use interface is the bottleneck in adapting nanospray to high throughput applications.

[09] Microfluidic device based electrospray sources for use in mass spectrometry have recently been developed; *see for example*, Oleschuk and Harrison, *Trends in Anal. Chem.*, **2000**, *19*, 379-388, and Licklider et al., *Anal. Chem.*, **2000**, *72*, 367-375. However, these methods utilize non-elastic microfluidic devices and require fabricating an electrospray nozzle directly on the microfluidic device or attaching a capillary electrospray emitter to the microfluidic device. Unfortunately, fabrication of an electrospray nozzle directly on the microfluidic device increases the manufacturing complexity, the production time and the cost. Methods for attaching a capillary electrospray emitter to current microfluidic devices also have severe limitations. For example, the junction between the microfluidic device and the electrospray nozzle emitter requires a tight seal to avoid fluid sample leakage. More significantly, it is difficult to attach an electrospray emitter to non-elastic microfluidic device without introducing a certain amount of void volume. Furthermore, the electrospray emitter must be carefully attached to the microfluidic device making mass production using batch processes difficult.

[10] Moreover, in these microfluidic devices the flow of fluid is typically electroosmotically driven or driven by applying pressure directly on the inlet layer of the microfluidic devices. These fluid flow methods further limit the utility of these microchips. For example, use of electroosmotic flow is incompatibility with many buffer systems, may cause molecular dissociation, and molecules can be damaged or degraded due to exposure to electric fields. Most importantly the ionic buffers required to drive electroosmotic flow interfere with electrospray ionization and limit its usefulness. The use of electric fields is also incompatible with applications that demand the use of non-aqueous solvents.

[11] Therefore, there is a need for a microfluidic device which comprises a means for providing a fluid sample to an analytical device. There is also a need for a microfluidic device in which a readily available electrospray emitter can be easily attached. There is also a need for a microfluidic device which does not require

electroosmotic flow or electrophoresis or a direct application of pressure on the inlet layer of the microfluidic device.

SUMMARY OF THE INVENTION

[12] The present invention provides a microfluidic apparatus for introducing a fluid sample into the injection port of an analytical device and a method for using the same. The microfluidic apparatus of the present invention comprises a microfluidic device and an integrated sample injection capillary which is in fluid communication with a flow channel of the microfluidic device. In one particular embodiment, the sample injection capillary forms a hermetic seal with the microfluidic device to allow the microfluidic device to introduce the fluid sample from the fluid channel to the injection port of the analytical device.

[13] The microfluidic device comprises an elastomeric block comprising a fluid inlet and a fluid outlet, and a fluid flow channel within the elastomeric block in fluid communication with the fluid inlet and said fluid outlet. When the cross section area of the fluid flow channel is significantly smaller than the cross section area of the sample injection capillary, the microfluidic device can optionally further comprises a sample injection capillary adapter layer proximal to the fluid outlet such that the sample injection capillary adapter layer has a larger cross sectional area than the cross sectional area of the fluid flow channel. In this manner, the sample injection capillary can be more easily accommodated by, i.e., inserted into or integrated within, the fluid outlet layer of the microfluidic device.

[14] The elastomeric block can optionally further comprise one or more control channels and one or more deflectable elastomeric layers located between the control channels and the fluid flow channel. The deflectable elastomeric layers can be actuated, i.e., deflected downward into the fluid flow channel, by a variety of means, *infra*, to affect the flow of the fluid sample within the fluid flow channel. In one embodiment, sequential actuation of the deflectable elastomeric layers produces a peristaltic pump action which causes the fluid sample to flow within the fluid flow channel.

[15] The microfluidic device can also further comprise an integrated sample preparation channel in fluid communication with the fluid flow channel. The sample preparation channel is capable of conducting a chemical reaction (e.g., combinatorial synthesis), chemical assay, chemical separation or other fluid sample preparation processes within the microfluidic device. In this manner, the fluid sample can be

prepared within the microfluidic device prior to being analyzed. In one embodiment, the sample preparation channel comprises a rotary fluid flow channel which is capable of forming a closed loop system within the elastomeric block by actuation of appropriate deflectable elastomeric layer(s), and a means for circulating the fluid sample within the rotary fluid flow channel. One method for circulating the fluid sample within the rotary fluid flow channel comprises a plurality of the control channels, and deflectable elastomeric layers described above. In this manner, actuating the deflectable elastomeric layers in a predetermined sequence causes flow of the fluid sample within the rotary fluid flow channel. The microfluidic device can optionally comprise a plurality of the sample preparation channel. In this manner, a variety of fluid sample preparation processes can be conducted within a single microfluidic device. Exemplary sample preparation processes include conducting a chemical reaction, conducting an assay, degrading a peptide or protein, conducting a chemical analysis, extraction of analytes from solvents, extraction of analytes from bodily fluids, concentration of sample analytes, affinity purification of an analyte, digesting a nucleic acid, carbohydrate, lipid or other molecule or mixture of molecules, separation, cell growth (mammalian, bacterial or parasite) and combinations thereof.

[16] In one particular embodiment, the fluid sample to be analyzed is prepared within the sample preparation channel by a combinatorial synthesis such that the fluid sample comprises an array of polymers derived from a monomer. Such combinatorial synthesis are useful in producing an array of nucleotides, amino acids, carbohydrates, lipids, and other precursor for combinatorial synthesis.

[17] In another embodiment, the fluid sample to be analyzed is prepared within the sample preparation channel by a receptor or an enzyme binding assay.

[18] Still in another embodiment, the fluid sample to be analyzed is prepared within the sample preparation channel by binding a target molecule to an array of oligonucleotides, peptides, proteins, oligosaccharides or small molecules that are covalently attached to the fluid flow channel(s) of the microfluidic device or vice versa.

[19] Yet in another embodiment, the fluid sample to be analyzed is prepared within the sample preparation channel by an enzymatic degradation of a protein, peptide, oligonucleotide, carbohydrate, lipid, small molecule or mixtures thereof.

[20] Another aspect of the present invention provides a method for producing a microfluidic sample injection apparatus described above comprising providing a first and a second elastomeric layers and forming a seal between the first and the second

elastomeric layers. Each of the first and the second elastomeric layers has a top surface and a bottom surface. The top surface of the first elastomeric layer has a microfabricated recess which when combined with the second elastomeric layer forms the microfluidic channel. The top surface of the second elastomeric layer also can optionally have a microfabricated recess for integrating the sample injection capillary. Such a sample injection capillary recess serves a variety of functions including placement of the sample capillary prior to attaching two elastomeric layers. In addition, the sample injection capillary recess provides larger cross sectional area for sample injection capillary adapter layer relative to the fluid flow channel.

[21] The sample injection capillary can be placed within a recess of one of the elastomeric layers prior to attaching two elastomeric layers together and forming a seal on the interface between the first and the second elastomeric layers. Alternatively, the two elastomeric layers can be bonded together to form a microfluidic device prior to attaching the sample injection capillary, e.g., by insertion.

[22] The elastomeric layers are preferably constructed from a mixture of two or more, preferably two, monomers. In this manner, one elastomeric layer can be constructed with an excess of a first monomer on its top surface and the other having an excess amount of the second monomer on its top surface. The two elastomeric layers can then be bonded together simply by attaching two top surfaces together and providing a polymerization reaction condition.

[23] Methods for producing the microfluidic sample injection apparatus of the present invention can also include inserting at least a layer of a conducting metal through the elastomeric block into the fluid flow channel proximal to the fluid outlet. In this manner, when an electrospray voltage is applied to the conducting metal an ionized mist is generated from the fluid sample in the fluid flow channel. Alternatively, the sample capillary comprises a tip, preferably a tapered tip, which is coated with a conductor metal. In such embodiment, the electrospray voltage is applied to the sample capillary tip to create an ionized mist from the fluid sample as it exits the microfluidic apparatus.

[24] Still another aspect of the present invention provides a method for analyzing a fluid sample using an analytical device which is operatively integrated with the microfluidic sample injection apparatus described herein. The analysis method generally involves introducing the fluid sample into a sample injection port of the analytical device using the microfluidic sample injection apparatus of the present invention and analyzing the fluid sample using the analytical device.

[25] The analytical device can be any conventional analytical devices known to one skilled in the art. Typically, the analytical device is selected from the group consisting of a UV/VIS spectrometer, fluorescence spectrometer, IR spectrometer, gas chromatographic device, liquid chromatographic device, NMR device, mass spectrometer and a combination thereof. Preferably, the analytical device is a mass spectrometer. More preferably, the analytical device is an electrospray ionization mass spectrometer or a nanospray mass spectrometer.

[26] The microfluidic sample injection apparatus of the present invention has significant advantages in both the sample preparation and sample delivery (in scales of nL/min). For example, in sample preparation, the combination of miniaturized valves and pumps on top of the fluid flow channel(s) allows one to conduct complex sample preparation processes, thereby circumventing shortcomings (some of which are described above) of electroosmotically driven microfluidic devices.

[27] Other benefits of the microfluidic sample injection apparatus of the present invention include reduced manufacturing and operating costs, reduced resource consumption, reduced waste production, and increased throughput (e.g., both by speeding up sequential, individual runs and also by implementing parallel processing). Further advantages of microfluidic devices of the present invention include adaptation of traditional liquid chromatography (i.e., LC) packing materials that enable separations to be permitted on the device. The sample processing and biochemical analysis provided by the present invention create "flow" or on-line systems, which can be adapted to high throughput methods.

[28] Thus, in one particular embodiment of the present invention, an integrated system of microfluidic device and ESI-MS (i.e., chip-ESI-MS) is used to process and then deliver nanoliter or picoliter scale samples with a uniform low sample flow rate (e.g., nL/min) for direct analysis of the fluid sample which has been prepared using the microfluidic device.

DEFINITIONS

[29] The terms "elastic block" and "elastic layer" are used interchangeably herein and refer to a material which can be deformed by applying pressure. Preferably, the Young's modulus of the elastic layer is from about 1 Pa to about 1 TPa, preferably from about 10 Pa to about 100 GPa, more preferably from about 20 Pa to about 1 GPa, still more preferably from about 50 Pa to about 10 MPa, and most preferably between

about 100 Pa to about 1 MPa. However, elastomeric materials having a Young's modulus outside of these ranges can also be utilized depending on the needs of a particular application.

[30] Unless otherwise stated, the term "liquid chromatography device" includes low-performance liquid chromatography devices (LPLC), medium-performance liquid chromatography devices (MPLC), and high-performance liquid chromatography devices (HPLC).

[31] Unless otherwise stated, the term "electrospray mass spectrometer" refers to electrospray ionization mass spectrometers, including nanoelectrospray mass spectrometers.

[32] The term "electrospray" refers to a method of generating a very fine liquid aerosol (i.e., mist) through electrostatic charging. Such methods are well known to one of ordinary skill in the art. Briefly, a plume of liquid droplets is generated by electrically charging a volume of liquid to a high voltage. The liquid becomes unstable as it is forced to hold more and more charge. When the liquid reaches a critical point (i.e., at critical charge/volume ratio), at which it can hold no more electrical charge, it rapidly dissociates (i.e., blows apart) into a cloud of tiny, highly charged "daughter" droplets. These tiny daughter droplets then fly towards detector which typically has opposite charge or ground potential. As droplets fly about, solvent molecules evaporate from their surface and the daughter droplets can further dissociate due to increased charge/volume ratio.

[33] The term "nanoelectrospray mass spectrometer" refers to mass spectrometers having a low sample fluid flow rate. Nanoelectrospray mass spectrometers have sample fluid flow rate in the range of from about 1 nL/min to about 150 nL/min, and preferably from about 20 nL/min to about 50 nL/min.

[34] The term "directly" as used in reference delivering or introducing a fluid sample from a microfluidic sample injection apparatus to an analytical device refers to a method for introducing a fluid sample to an analytical device without any intervening manual manipulation of the fluid sample. In particular, the fluid sample leaving the microfluidic sample injection apparatus enters the injection port of the analytical device directly.

[35] The terms "injected" and "introduced" are used interchangeably herein and refer to providing the fluid sample into the analytical device for analysis.

[36] The term "cross section" refers to the cross-section of a channel that can be a circle, oval, ellipse, rectangle, square, triangle or other geometrical shapes.

[37] The terms "capillary nozzle" and "capillary tip" are used interchangeably herein and refer to a tip of a capillary tube that can be tapered or non-tapered.

[38] "Tube" refers to a hollow elongated device having a two open ends. The cross section of a tube can be circular of non-circular. Preferably, the cross section of the tube is circular.

[39] "Circular" refers to a circle-like shape. Thus, the term circular includes oval, circle, and ellipse.

[40] "Capillary" refers to a tube with a small inner diameter, i.e., < 1 mm. Preferably, the inner diameter of the capillary nozzle is from about 1 μm to about 100 μm, more preferably from about 10 μm to about 50 μm, and most preferably from about 10 μm to about 20 μm.

[41] The term "analyte" refers to a particular compound which is to be analyzed by the analytical device.

[42] The term "rotary" refers to a configuration in the fluid flow channel which allows circulation of a fluid within a confined region or section of the fluid flow channel.

[43] The term "channel" refers to an empty space within the elastomeric block in which a fluid can be introduced. Preferably, a liquid is introduced in a fluid channel and a gas is introduced in a pressure channel.

[44] "Fluid" refers to a gas medium or, preferably, a liquid medium.

BRIEF DESCRIPTION OF THE DRAWINGS

[45] Figure 1A is a schematic illustration of a microfluidic device comprising a means for delivering a fluid sample directly to an injection port of an analytical device;

[46] Figure 1B is a schematic illustration of an analytical apparatus comprising a microfluidic device having an electrospray capillary interconnected to a mass spectrometer;

[47] Figure 2A is a front view of a microfluidic device comprising a fused silica capillary nozzle which can be used as an electrospray source;

[48] Figure 2B is a top view illustrating a layer of microfluidic device which comprises pumps (dotted line) on a layer above the fluid flow channel (solid line);

[49] Figure 2C is a close-up view of one particular embodiment of an interface between a fluid flow channel in microfluidic device and a capillary tube which is used as a means to deliver a fluid sample to an analytical device (not shown);

[50] Figures 3A and 3B are a schematic illustration of creating an arcuate (e.g., rounded) fluid flow channel elastomer using a photoresist mold;

[51] Figure 4A is a schematic illustration of an elastomeric peristaltic pump located above a fluid flow channel;

[52] Figure 4B is a graph showing pump rate (i.e., fluid flow rate) versus frequency of peristaltic pump of Figure 4A;

[53] Figure 5 is a schematic illustration of a fluid flow channel comprising a sample preparation chamber and reagent injection sites;

[54] Figure 6A is a front view of the first elastic layer integrated with a capillary nozzle;

[55] Figure 6B is a side cross-sectional view of the first elastic layer fitted with a capillary nozzle with dead volume in between the fluid channel and the capillary nozzle;

[56] Figure 6C is a side cross-sectional view of the first elastic layer fitted with a capillary nozzle having a tapered fitting end which reduces the amount of dead volume;

[57] Figure 7A is a perspective view of the first elastic layer having a rectangular cross-section fluid flow channel;

[58] Figure 7B is a cut-away view along 1-1' of Figure 7A showing a tapered layer of fluid flow channel which is designed to reduce the amount of dead volume between the capillary nozzle and the fluid flow channel;

[59] Figure 7C is a front view of the first elastic layer fitted with a capillary nozzle illustrating a possible gap formation between the capillary nozzle and the fluid flow channel;

[60] Figure 8 is another embodiment of attaching a capillary nozzle to a microfluidic device of the present invention through the Z-axis;

[61] Figure 9A is a schematic illustration of one embodiment of using a capillary nozzle as an electrospray;

[62] Figure 9B is a close-up view of the capillary nozzle of Figure 9A;

[63] Figure 9C is a schematic illustration of another embodiment of using a capillary nozzle as an electrospray; and

[64] Figure 9D is a close-up view of the capillary nozzle of Figure 9C.

DETAILED DESCRIPTION

[65] The present invention will be described with regard to the accompanying drawings which assist in illustrating various features of the invention. In this regard, the

present invention generally relates to a microfluidic sample injection apparatus for providing a fluid sample from to an analytical device and a method for producing and using the same. In the accompanying drawings the same numbers in different drawings represent identical elements. The drawings are provided for the purpose of illustrating the practice of the present invention and do not constitute limitations on the scope thereof.

[66] One aspect of the present invention provide a microfluidic sample injection apparatus comprising a microfluidic device **100** and an integrated sample injection capillary **14** which provides a fluid sample into the injection port **114** of an analytical device **120**. The analytical device **120** can be any device which is used for analyzing a chemical compound. While the sample can be in a pure form (i.e., neat), microfluidic devices of the present invention are particularly useful for analyzing analytes that are in a solution.

[67] Preferably, the analytical device **120** is a selected from the group consisting of a UV/VIS spectrometer, fluorescence spectrometer, IR spectrometer, gas chromatographic device, liquid chromatographic device, NMR device, mass spectrometer and combinations thereof. More preferably, the analytical device **120** is a mass spectrometer. And most preferably, the analytical device **120** is an electrospray or a nanospray mass spectrometer.

[68] In one embodiment, the apparatus of the present invention comprises a microfluidic device **100** and a fluid sample introducing means **124**. The fluid sample introducing means **124** can include any device that allows delivery of the fluid sample from the microfluidic device **100** to the analytical device **120**. Exemplary fluid sample delivery devices include the outlet port of fluid flow channel on the microfluidic device **100**; a capillary nozzle **14** (such as electrospray nozzles); needles, and other devices which can be integrated with the microfluidic device **100**. Preferably, the fluid sample introducing means **124** is a capillary **14**.

[69] Microfluidic devices of the present invention are capable of delivering a very minute amounts of samples to the analytical device, thereby increasing the sensitivity of the analytical device **120**. In particular, microfluidic devices of the present invention are capable of providing a fluid sample to the analytical device at a flow rate of from about 1 nL/min to about 200 nL/min, preferably from about 10 nL/min to about 50 nL/min, and more preferably from about 10 nL/min to about 20 nL/min.

[70] While the present invention is generally described in reference to microfluidic devices for preparing and/or providing fluid samples to electrospray and

nanospray mass spectrometers, it should be appreciated that the present invention is not limited to such. For example, microfluidic devices of the present invention can be integrated with a LC-mass spectrometer, GC-mass spectrometer, other liquid or gas chromatography devices, IR spectrometer, UV/VIS spectrometer, fluorescence spectrometer, or the like by using a capillary, needle, or some other fluid sample delivery means which provide a non-aerosol (i.e., mist) liquid samples to analytical devices.

[71] One aspect of the present invention provides a microfluidic device **100** comprising a first elastomeric block **25**, a fluid flow channel **18** within the first elastomeric block **25**, and a fluid sample providing means **124**. See, for example, Figs. 1A-2C. As can be seen in Figs. 2A and 2C, the first elastomeric block **25** can be made from attaching a first elastic layer **10** and a second elastic layer **20**. The third elastic layer **30** is optional. However, when the third elastic layer **30** is present, preferably it comprises a control channel and a deflectable elastomeric layer located between the fluid flow channel and the control channel, as discussed in detail below. Preferably, the fluid sample is introduced directly from the fluid flow channel **18** to an analytical device **120**. Methods for producing microfluidic devices comprising an elastic layer is generally described in U.S. Patent Application Serial No. 09/605,520, filed on June 27, 2000, which is incorporated herein by reference in its entirety. The first elastomeric block **25** of microfluidic devices of the present invention preferably comprises at least two elastic layers, the first elastic layer **10** and a second elastic layer **20**. In this embodiment, the fluid flow channel **18** is formed at the interface of these two elastic layers. This is particularly advantageous when forming a fluid flow channel having a circular cross-section.

[72] Preferably the inner diameter of the sample injection capillary **14** is from about 1 μm to about 100 μm, more preferably from about 10 μm to about 50 μm, and most preferably from about 10 μm to about 20 μm. The outer diameter of the capillary nozzle **14** dependents on the width (e.g., diameter) of the fluid flow channel **18** or the layer of fluid flow channel which is integrated with the capillary nozzle, i.e., sample injection capillary adapter layer.

[73] It is preferred that the inner diameter of the sample injection capillary **14** be substantially similar to the width of fluid flow channel **18**, as this diameter to width matching allows minimal fluid flow disruption and/or pressure differential between the fluid flow channel **18** and the sample injection capillary **14**. Typically, the outer diameter

of the sample injection capillary **14** is larger than the width of fluid flow channel **18**. Thus, in order to provide a substantially similar width, the layer of fluid flow channel **18** which integrates the sample injection capillary **14** (i.e., sample injection capillary adapter layer) is constructed such that its width is substantially similar to the outer diameter of the sample injection capillary **14**. It is preferred, however, that the volume of the sample injection capillary adapter layer be slightly smaller than the volume occupied by the outer dimension of the sample injection capillary. Such a volume difference provides a "snug" fit or a hermetic seal between the sample injection capillary and the fluid flow channel or the sample injection capillary adapter layer. This is particularly useful in microfluidic devices of the present invention as they have an elastic layer which can expand to accommodate the sample injection capillary **14**. It should be appreciated that the amount of expansion possible by the elastomeric layer depends on the particular nature of the material used. Alternatively, an adhesive can be used to secure the sample injection capillary **14** within the flow channel **18** or the sample injection capillary adapter layer.

[74] It has been found by the present inventors that to achieve a minute fluid sample flow rate (e.g., in nL/min scale), a microfluidic device that is constructed using multi-layer soft lithography having one or more elastomeric pumps **34** is particularly useful. See for example, Unger et al, *Science*, **2000**, *288*, 113-116, and U.S. Patent Application Serial No. 09/09/605,520, filed June 27, 2000, all of which are incorporated herein by reference in their entirety. Thus, preferred microfluidic devices of the present invention can further comprise a second elastic layer (i.e., a third elastomeric layer) **30** which comprises one or more pump and/or valve systems which are comprised of one or more control channels and deflectable elastomeric layer located between the fluid flow channel and the control channel. These pumps and valves are capable of controlling the flow fluid sample within the fluid flow channel **18** by selectively actuating deflectable elastomeric layer(s), i.e., by closing and opening particular sections of fluid flow channel **18**. Furthermore, the rate of fluid flow within the fluid flow channels can be controlled by the actuation rate of these deflectable elatomeric layers (i.e., pumps). These pumps are capable of providing sample flow rate of pico- to nanoliter per minute though the fluid flow channel **18**. Such a low fluid sample flow rate provides injection of a minute quantities of fluid sample to the analytical device **120** through the sample injection capillary **14**. For example, using the fluid channel arrangement shown in Figure 4A, where the fluid flow channels are 100 μm wide and 10 μm high, with 30 μm gap (i.e.,

deflectable elastomeric layer) between the fluid channel **18** and the control channels **50**, the rate of fluid sample flow through the fluid channel **18** is measured as a function of peristaltic pump **34** frequency (e.g., actuation rate of each deflectable elastomeric layer). Peristalsis is actuated by the pattern 101, 100, 110, 010, 011, 001, where 0 an 1 indicate "valve open" and "valve closed," respectively. Figure 4B shows the pump rate (nL/s) per peristalsis frequency. By reducing the frequency of the pump **34** and/or the dimensions of the fluid channel **18**, one can easily adjust the fluid sample flow rate within the fluid channel **18** to pico- to nanoliter per minute.

[75] Preferably, microfluidic devices of the present invention are capable of delivering from about 0.5 nL/min to about 200 nL/min of fluid sample to the analytical device, more preferably from about 10 nL/min to about 50 nL/min, and most preferably from about 10 nL/min to about 20 nL/min. These minute delivery rate allows a very minute quantity of analyte in the fluid sample to be injected into the analytical device **120** over a much longer period of time than otherwise possible using conventional means. This constant stream of the fluid sample over a relatively long period of time allows more accurate analysis of minute quantities of analytes than currently possible.

[76] Another advantage of pump-driven elastomeric microfluidic devices of the present invention over current microfluidic devices that use electrokinetic flow means is that electric fields are not required to drive the flow of the solvent, therefore the rate of fluid flow is composition independent. Moreover, electrokinetic flow requires a relatively high salt concentration buffer solution to affect fluid sample flow. The inclusion of high salt concentrations in the buffer creates problems with ionization in analytical devices, such as electrospray-ionization mass spectrometer (i.e., ESI-MS), and causes a significant background noise. Since microfluidic devices of the present invention do not require electric fields, they eliminate the need for a high salt concentration buffer solution, thereby reducing the background noise and increasing the sensitivity of the analytical device. Furthermore, the lack of requirement for a high salt concentration buffer solution also allows the use of non-aqueous solvents, thereby greatly extending the field of application of these devices.

[77] Microfluidic devices of the present invention can also comprise a sample preparation chamber (e.g., item **50** of Fig. 5) in fluid communication or within the fluid flow channel **18**. In this manner, the fluid sample can be prepared within the microfluidic device **100** and injected into the analytical device **120**. The sample preparation chamber can be any configuration which allows preparation of the fluid sample to be analyzed.

For example, the sample preparation chamber can be an array of fluid flow channels and control channels (i.e., pumps and valves) which can be used in combinatorial synthesis. Exemplary microfluidic devices for combinatorial synthesis are disclosed in Patent Application entitled "Combinatorial Synthesis System," filed on October 3, 2000, by R. Michael van Dam, Marc Unger and Stephen Quake, and further identified as Attorney Docket No. 020174-001600US, the disclosure of which is incorporated herein by reference in its entirety. As shown in Figure 5, the sample preparation chamber can include or be a rotary fluid flow channel **50** and a means for circulating a fluid (e.g., by using circulation pumps **34'** and closing valves **42A** and **42B**, i.e., control channels) within the rotary fluid flow channel **50**. The rotary fluid flow channel **50** can be used to conduct a chemical reaction, an assay, protein degradation, separation, or other sample preparations processes.

[78] For example, chemical reaction can be conducted by introducing reagents through fluid flow channels **18** using one or more fluid inlets, e.g., sample or reagent injection ports **46A** and **46B** (see Figure 5). The reagents are then pumped by the pump **34** (e.g., see Figure 2B) which can be located anywhere along the fluid flow channel **18**. These reagents can be admixed and allowed to react for a period of desired time by "holding" the reaction mixture within the rotary fluid flow channel **50** by closing the valves **42A** and **42B** and optionally circulating the reaction mixture within the rotary fluid channel using the circulation pump **34'**. Once the chemical reaction is complete (or after a certain period of time) the valves **42A** and **42B** are opened and the reaction mixture is pumped through the fluid flow channel **18** by the pump **34** into the capillary nozzle **14**, which is connected to a sample injection port **114** of the analytical device **120**. The reaction mixture (i.e., fluid sample) is then injected into the analytical device **120** and the reaction product(s) are analyzed.

[79] In an assay sample preparation, desired compounds, enzymes or cells are immobilized on the surface of sample preparation chamber. Methods for immobilizing these materials on a solid support containing functional groups such as hydroxides or amines is well known to one of ordinary skill in the art. For example, U.S. Patent Nos. 5,424,186 and 5,959,098, which are incorporated herein by reference in their entirety, describe immobilization of polymers such as oligonucleotides and peptides on solid support. If the inner surface of sample preparation chamber is hydrophobic, it can be functionalized with hydrophilic functional groups. For example, a hydrophobic elastomer surface can be treated with oxygen or water plasma to introduce hydroxy functional

groups, which can then be used to immobilize desired materials. After desired materials are immobilized, a mixture of compounds is then introduced into the sample preparation chamber. The mixture of compounds can be exposed to the immobilized material for desired time by circulating the mixture within the sample preparation chamber, or alternatively the mixture of compounds can be exposed to the immobilized material by allowing the mixture to simply flow through the sample preparation chamber (in which case the sample preparation chamber need not be a rotary fluid flow channel). The appropriate target compound(s) then bind to the immobilized material while other compounds are washed away. After removing non-binding compounds, the bound compounds can be released from the immobilized material, e.g., by adding more strongly binding competitive binding compounds or by denaturing the enzyme to release the bound compounds. The released compounds can then be injected into the analytical device directly and analyzed.

[80] The sample preparation chamber can also be used to degrade proteins into smaller components (e.g., oligonucleotides or amino acids) for analysis. For example, one can integrate a tryptic proteolysis of a peptide on the microfluidic device **100** and inject the resulting sample into the analysis device **120** for analysis. For a representative illustration of a tryptic proteolysis of a peptide see Xue et al., *Rapid Commun. Mass Spectrom.*, **1997**, *11*, 1253, which is incorporated herein by reference in its entirety. In addition, a peptide can be degraded in the sample preparation chamber by immobilizing an enzyme, as described above, which is capable of degrading the peptide and introducing the peptide into the sample preparation chamber. The degraded peptide components can then be effused into a mass spectrometer for analysis. Such peptide sequencing using a mass spectrometer is well known to one of ordinary skill in the art. See for example, Shevchenko et al., *Rapid Commun. Mass Spectrom.*, **1997**, *11*, 1015-1024, which is incorporated herein by reference in its entirety. Briefly, Shevchenko et al. describe a rapid peptide sequencing using a combination of nanoelectrospray quadrupole/time-of flight mass spectrometer and isotopic labeling of the peptide. By analyzing the mass spectrum pattern of fragments of peptides and comparing the results with known database of peptide mass spectrum patterns, Shevchenko et al. were able to sequence a peptide relatively quickly.

[81] Alternatively, the sample preparation chamber can be a DNA sorter as disclosed by Chou et al., *Proc. Natl. Acad. Sci.*, **1999**, 11-13, or a cell sorter as disclosed in PCT Patent Application Publication No. WO 99/61888, which are incorporated herein

by reference in their entirety. Thus, compounds can be sorted based on, e.g., a particular fluorescence wavelength and analyzed by the analytical device.

[82] In addition, by having a layer of the fluid flow channel filled with affinity sieves or similar chromatography material, a mixture of compounds can be separated and each compounds can be analyzed separately.

[83] It should be appreciated that one or more of the above described sample preparation steps can be combined sequentially to provide a variety of sample preparation combinations. For example, sample preparation step can include preparing (i.e., synthesizing) compounds in one sample preparation chamber which is connected to another sample preparation chamber for assaying the compounds, e.g., for enzyme binding. In this manner, a variety of manipulations can be conducted in a single microfluidic device or a combination of microfluidic devices before injecting the analyte into the analytical device for analysis (e.g., identification).

Methods of Fabricating Microfluidic Devices

[84] One exemplary method of fabricating microfluidic devices of the present invention is provided herein, which is similar to methods disclosed in U.S. Patent Application Serial No. 09/605,520, which was previously incorporated by reference. It is to be understood that the present invention is not limited to fabrication by this method. Rather, other suitable methods of fabricating the present microstructures, including modifying the present methods, are also contemplated to be within the scope of the present invention.

[85] Figures 3A and 3B illustrate sequential steps of a preparing a rounded (i.e., circular cross-section) fluid flow channel. Preferably, channels are molded in each layer of the elastomer using soft lithography. A thin layer of photoresist **64** is spin coated on to a wafer **60**. The photoresist is exposed using a high transparency film as a mask (not shown) with desired pattern. The exposed photoresist is then developed to provide a mold (for clarity only one flow channel mold **64A** is shown). The height of flow channel mold **64A** depends on the thickness of the photoresist. If desired, multiple layers of photoresist can be applied to achieve the desired thickness, using intermediate 'hard bake,' which generally involves heating (e.g., to about 120 °C) to fix channel structures in one part of the device prior to creation of the bottom layer **10** or a second layer **30**. This approach can be used to create regions with different channel depth in different parts of the same device.

[86] Typically, the exposure and development of a photoresist results in a trapezoidal shaped mold. The photoresist is then heated (e.g., at 200 °C for about 30 minutes) to "reflow" the photoresist, thereby producing a rounded flow channel mold **64B**. This "rounding" facilitates sealing of capillaries of different dimensions within the device. A layer of elastomer **20** (i.e., top layer of the first elastic layer) is then spin coated on to the mold, as shown in Figure 3B. After curing, the elastomer is removed from the mold to provide a recess which becomes a part of the flow channel **18**. A complimentary bottom layer elastomer **10** is produced and combined with the top layer **20** to produce a first elastomeric block **25**. A second elastic layer **30** comprising pump **34** and valve **42** systems is then produced as a single layer and bonded together with (i.e., affixed on top of) the first elastomeric block **25**.

[87] Upon casting in elastomer, such as GE Silicones RTV615 or Dow Corning Sylgard, a channel is created whose depth is dependent on the thickness of the photoresist upon the wafer. The sample injection capillary **14** is formed by placing a sample injection capillary (e.g., a drawn silica capillary) in the flow channel **18**, so that the sample injection capillary **14** sits, i.e., placed, within the flow channel **18** and extends beyond the edge of the microfluidic device **100**. Typically, the distance of the sample injection capillary extension beyond the edge of the microfluidic device **100** is from about 50 μm to about 5 mm, preferably from about 100 μm to about 2 mm, and more preferably from about 100 μm to about 1 mm. However, the distance of the extension can be longer depending on a particular application. The sample injection capillary **14** is sealed within two elastomeric layers (Figures 6A and 6B). It can be sealed either directly by baking together the two elastomeric layers of partially cured elastomers or by incorporation of uncured elastomer (e.g., RTV) during the final bake (i.e., curing) stage.

[88] Fluids are designed to flow in the middle of the two layers (i.e., in between the first and the second elastomeric layers **10** and **20**) of this device. The alignment of the sample injection capillary **14** between the two layers and its juxtaposition with the fluid channel **18** can create a partial occlusion of the capillary if the capillary is perfectly centered between the layers. Better alignments can be achieved by creating an offset in the depths (i.e., height) of two layers of the channels between which the sample injection capillary is fitted. For example, if the depth of the photoresist for the recess of the first and the second elastomeric layers is 5 microns each, then a sample injection capillary with a ten-micron internal diameter can be accommodated without a significant offset.

[89] The layer of fluid flow channel **18** which becomes integrated with the sample injection capillary **14** can be configured such that the fluid sample flows directly from the microfluidic device **100** to the analytical device (e.g., mass spectrometer). Additional features patterned in photoresist may be necessary to reduce potential dead volume **16** at the junction between the sample injection capillary **14** and the fluid flow channel **18**.

[90] An alternative method of reducing the amount of dead volume is illustrated using a tapered capillary as shown in Figure 6C. This configuration is compatible with commercially available drawn silica capillaries and custom-drawn capillaries. The dimensions of the capillaries that can be accommodated in this configuration include, but are not limited to, capillaries with internal diameters of from about 1 μm to about 100 μm and outer diameters of from about 20 μm to about 360 μm.

[91] In order to create 'pump' and 'valve' features within the microfluidic device **100**, a third elastomeric layer (i.e., layer) **30** having 'control line' features (for pumps and valves) is bonded on top of the elastomeric block comprising the fluid flow channel. This third elastic layer is prepared using a similar process for the above described first and second elastomeric layers of the elastomeric block. Typically, the third elastomeric layer is baked (or cured) together with the elastomeric block to create the final monolithic microfluidic device.

[92] The sample injection capillary **14** can be sealed within the microfluidic device **100**, by a variety of processes. For example, the sample injection capillary **14** can be sealed during baking together of the first and the second elastomeric layers **10** and **20**. Alternatively, as shown in Figures 7A-7C, the sample injection capillary **14** is 'push-fit' into the microfluidic device between two elastomeric layers, thereby creating an instant seal. The dimensions of the push-fit envelope are chosen to accommodate the diameter of the sample injection capillary **14**. For example, an envelope of about 200 μm width and about 15 μm in height has a perimeter of 430 microns. A sample injection capillary with 100 μm outer diameter has a circumference of 314 μm. The seal can be further secured by incorporation of uncured elastomer (e.g., RTV) in the envelope between the two layers (e.g., areas **22A** and **22B**). As shown in Figure 8, push fitting can also be used to incorporate a sample injection capillary that fits into the device in the 'Z' plane. One major advantage of push fitting is that sample injection capillaries can be easily interchanged if clogging occurs.

Application of high voltage for electrospray

[93] A high voltage applied to capillary nozzle causes ionization of molecules passing through it at atmospheric pressure and formation of a plasma stream that is accelerated into the analytical device (e.g., mass spectrometer). The system allows for both the sample preparation and sample delivery processes for the ESI-MS to be integrated on the microfluidic device.

[94] Typically application of voltages of from about 500 to about 5 kilovolts (kV) are required to create the electrospray. Two illustrative examples are shown in Figures 9A-9D. One method, as shown in Figures 9A and 9B uses a metallized (e.g., Pd or Gold) or metal coated capillary, which are commercially available. In this method, a high voltage device **110** applies voltage to sample injection capillary **14** having a metal coating **12** which creates electrospray **8**. Unfortunately, these types of capillaries have a limited life-span due to evaporation of the metallized layer that carries the charge. Another method uses an external metal sheath capillary as shown in Figures 9C and 9D. In this embodiment, a high voltage device **110** applies voltage to sample injection capillary **14** having a metal sheath **12'** to create electrospray **8**. This method has the added benefit that it can be built into a plastic housing for the device and the capillary itself can be used for more than one sample.

[95] Still another method (not shown) for creating an ionized mist from the fluid sample is to insert a conducting metal proximal (i.e., near) the fluid outlet layer of the microfluidic device near the interface between the fluid flow channel and the sample injection capillary. This conducting metal is then operatively interconnected with an electrospray voltage generator **110** to generate an ionized mist from the fluid sample that is flowing within the fluid flow channel. Typically the conducting metal is simply inserted through the elastomeric block such that the tip of the conducting metal is placed within the fluid flow channel.

Applications:

[96] The integrated microfluidic devices that comprise fluid flow channels, pumps and valves can be used in a variety of applications as discussed above. In addition, such devices can be used as nanoliter-scale fluid delivery devices for reliably delivering highly homogeneous, nanoliter volumes (e.g., from about 1 nL/min to about 200 nL/min) of fluid to the mass spectrometer interface. Such devices can also be used as nanoliter-scale devices which integrate sample purification, separation and processing, as

discussed in detail above. This reduces sample preparation cost, avoids sample cross contamination, and enables the application of mass spectrometry to other areas of interest, such as medical diagnostics. Furthermore, such devices can be interfaced with a robotic auto sampler to provide high throughput nanospray device. In one example of this device, multiple channels are created in the elastomer, each of which is operated by a single set of pumps and individually sampled using a control valve.

[97] Microfluidic devices of the present invention are useful in proteomics such as classic proteomics, e.g., identification and quantitation of unknown proteins identified using 1-D and 2-D gel electrophoresis, and functional proteomics, e.g., analysis of molecular interactions. In addition, microfluidic devices of the present invention are also useful in drug or target molecule discovery. NanoES MS/MS is the most powerful approach currently available, as it allows unambiguous protein and peptide fragment data to be queried against EST and genomic databases. For example, a protein identified as differentially expressed or with variable post-translational modification when two samples or tissues are compared can be identified by comparing the peptide sequences obtained by mass spectrometry against EST and genomic databases.

[98] Moreover, the identified nucleotide sequences, combined with the peptide sequences generated by mass spectrometry, can be used for cloning the protein, in downstream assay development, target validation. And as discussed in detail above, on-chip digestion (i.e., degradation) of proteins with proteases (for example using immobilized trypsin) and on-chip separations can also be achieved by using microfluidic devices of the present invention.

[99] In addition, microfluidic devices of the present invention can be used to assist in drug development by enabling unambiguous identification of metabolites in serum, urine, etc. Furthermore, microfluidic devices of the present invention can be used in ADME/PK (absorption-distribution-metabolism-excretion/pharmacokinetic) studies. Additionally, high throughput screening can be conducted directly by using the MS to provide assay readout.

[100] Other uses for microfluidic devices of the present invention include, but are not limited to, applications in genomics, e.g. high throughput genotyping, applications in analytical chemistry, on chip separations, on-chip combinatorial chemistry, and analysis of proteins in clinical diagnostics.

[101] In particular, the sample preparation chamber can be used for conducting a chemical reaction; conducting an assay; degrading a peptide or protein; conducting a

chemical analysis; extraction of analytes from solvents (aqueous/non-aqueous); extraction of analytes from bodily fluids; concentration of sample analytes; affinity purification of an analyte; digesting a nucleic acid, carbohydrate, lipid or other molecule or mixture of molecules; separation; and cell growth (mammalian, bacterial or parasite).

[102] In combinatorial synthesis, microfluidic devices of the present invention can us a monomer (i.e., starting material) that is selected from the group consisting of nucleotides, amino acid peptides, carbohydrates, lipids, and other precursors for combinatorial synthesis.

[103] The sample preparation step can also comprise binding a target molecule to an array of oligonucleotides, peptides, proteins, oligosaccharides, and small molecules (e.g., drugs).

Preferred Layer and Channel Dimensions:

[104] Microfabricated refers to the size of features of an elastomeric structure fabricated in accordance with an embodiment of the present invention. In general, variation in at least one dimension of microfabricated structures is controlled to the micron level, with at least one dimension being microscopic (i.e. below 1000 μm). Microfabrication typically involves semiconductor or MEMS fabrication techniques such as photolithography and spin coating that are designed for to produce feature dimensions on the microscopic level, with at least some of the dimension of the microfabricated structure requiring a microscope to reasonably resolve/image the structure.

[105] For integration with an electrospray capillary for use in a mass spectrometer, preferred width-to-depth ratios of a rectangular cross-section fluid flow channel **18** is from about 0.1:1 to about 100:1, more preferably from about 1:1 to about 50:1, still more preferably from about 2:1 to about 20:1, and most preferably from about 3:1 to about 15:1. For a circular cross-section fluid flow channel **18**, preferred diameter is from about 1 μm to about 500 μm, more preferably from about 1 μm to about 200 μm, and most preferably from about 3 μm to about 200 μm.

[106] The flow channels are not limited to these specific dimension ranges and examples given above, and can vary depending on a particular sample delivery means employed. For example, wider flow channels having a diameter in the order of about 1000 μm may be useful in other analytical device interface, such as HPLC or UV spectrometer.

[107] The total thickness of the first elastomeric block **25** also depends on a particular application. For use in electrospray-MS with nL/min sample deliver rate, the first elastomeric block layer **25** has thickness of from about 40 μm to about 10 mm, preferably from about 40 μm to about 5mm, and more preferably from about 40 μm to about 3 mm.

[108] Accordingly, the layer of elastomer separating the flow channel **18** and the control channel (e.g., pumps and valves) has a typical thickness of from about 0.01 μm to about 1000 μm, preferably from about 0.05 μm to about 500 μm, more preferably from about 0.2 μm to about 250 μm, still more preferably from about 1 μm to about 100 μm, yet still more preferably from about 2 μm to about 50 μm, and most preferably from about 5 μm to about 40 μm.

[109] The control channels that make up the pump and valve systems typically have rectangular cross-section for ease of fabrication. However, the cross-section is not limited to such shape. Preferably the width of control channels is from about 0.01 μm to about 1000 μm, preferably from about 0.05 μm to about 1000 μm, more preferably from about 0.2 μm to about 500 μm, still more preferably from about 1 μm to about 250 μm, and most preferably from about 10 μm to about 200 μm. The thickness of the third elastomeric layer **30** is from about 50 μm to several centimeters, preferably from about 0.1 μm to about 10 cm, more preferably from about 1 μm to about 5 cm, still more preferably from about 10 μm to about 2 cm, and most preferably from about 100 μm to about 10 mm.

Multilayer Soft Lithography Construction Techniques and Materials:

[110] Preferably, the first and the second elastomeric layers **10** and **20** (and between the first elastomeric block **25** and the third elastomeric layer **30**) are bonded together chemically, using chemistry that is intrinsic to the polymers comprising the patterned elastomer layers. Most preferably, the bonding comprises two component "addition cure" bonding.

[111] In a preferred aspect, the layers of elastomer are bound together in a heterogenous bonding in which the layers have a different chemistry. Alternatively, a homogenous bonding can be used in which all layers would be of the same chemistry. Thirdly, the respective elastomer layers can optionally be glued together by an adhesive

instead. In a fourth aspect, the elastomeric layers can be thermoset elastomers bonded together by heating.

[112] In one aspect of homogeneous bonding, the elastomeric layers are composed of the same elastomer material, with the same chemical entity in one layer reacting with the same chemical entity in the other layer to bond the layers together. In one embodiment, bonding between polymer chains of like elastomer layers can result from activation of a crosslinking agent due to light, heat, or chemical reaction with a separate chemical species.

[113] Alternatively in a heterogeneous aspect, the elastomeric layers are composed of different elastomeric materials, with a first chemical entity in one layer reacting with a second chemical entity in another layer. In one exemplary heterogenous aspect, the bonding process used to bind respective elastomeric layers together can comprise bonding together two layers of RTV 615 silicone. RTV 615 silicone is a two-part addition-cure silicone rubber. Part A contains vinyl groups and catalyst; part B contains silicon hydride (Si-H) groups. The conventional ratio for RTV 615 is 10A:1B. For bonding, one layer can be made with 30A:1B (i.e. excess vinyl groups) and the other with 3A:1B (i.e. excess Si-H groups). Each layer is cured separately. When the two layers are brought into contact and heated at elevated temperature, they bond irreversibly forming a monolithic elastomeric substrate.

[114] In an exemplary aspect of the present invention, elastomeric structures are formed utilizing Sylgard 182, 184 or 186, or aliphatic urethane diacrylates such as (but not limited to) Ebecryl 270 or Irr 245 from UCB Chemical.

[115] Alternatively, other bonding methods can be used, including activating the elastomer surface, for example by plasma exposure, so that the elastomer layers/substrate bond when placed in contact. For example, one approach to bonding together elastomer layers composed of the same material is set forth by Duffy et al, "Rapid Prototyping of Microfluidic Systems in Poly(dimethylsiloxane)", *Analytical Chemistry*, **1998**, *70*, 4974-4984, which is incorporated herein by reference in its entirety. This paper discusses that exposing polydimethylsiloxane (PDMS) layers to oxygen plasma causes oxidation of the surface, with irreversible bonding occurring when the two oxidized layers are placed into contact.

[116] Yet another approach to bonding together successive layers of elastomer is to utilize the adhesive properties of uncured elastomer. Specifically, a thin layer of uncured elastomer such as RTV 615 is applied on top of a first cured elastomeric layer.

Next, a second cured elastomeric layer is placed on top of the uncured elastomeric layer. The thin middle layer of uncured elastomer is then cured to produce a monolithic elastomeric structure. Alternatively, uncured elastomer can be applied to the bottom of a first cured elastomer layer, with the first cured elastomer layer placed on top of a second cured elastomer layer. Curing the middle thin elastomer layer again results in formation of a monolithic elastomeric structure.

Suitable Elastomeric Materials:

[117] Allcock et al, *Contemporary Polymer Chemistry*, 2nd Ed. describes elastomers in general as polymers existing at a temperature between their glass transition temperature and liquefaction temperature. Elastomeric materials exhibit elastic properties because the polymer chains readily undergo torsional motion to permit uncoiling of the backbone chains in response to a force, with the backbone chains recoiling to assume the prior shape in the absence of the force. In general, elastomers deform when force is applied, but then return to their original shape when the force is removed. The elasticity exhibited by elastomeric materials may be characterized by a Young's modulus. Elastomeric materials having a Young's modulus of between about 1 Pa – 1 TPa, more preferably between about 10 Pa – 100 GPa, still more preferably between about 20 Pa – 1 GPa, yet more preferably between about 50 Pa – 10 MPa, and most preferably between about 100 Pa – 1 MPa are useful in accordance with the present invention. It should be appreciated, however, elastomeric materials having a Young's modulus outside of these ranges can also be utilized depending upon the needs of a particular application.

[118] Microfluidic devices of the present invention can be fabricated from a wide variety of elastomers. In an exemplary aspect, the first, second and third elastomeric layers **10**, **20** and **30** are preferably fabricated from silicone rubber. However, other suitable elastomers may also be used.

[119] In an exemplary aspect of the present invention, microfluidic devices are fabricated from an elastomeric polymer such as GE RTV 615 (formulation), a vinyl-silane crosslinked (type) silicone elastomer (family). However, microfluidic devices of the present invention are not limited to this one formulation, type or even this family of polymer; rather, nearly any elastomeric polymer is suitable. In the case of multilayer soft lithography, preferably layers of elastomer are cured separately and then bonded together. This scheme requires that cured layers possess sufficient reactivity to bond together. Either the layers can be of the same type, and are capable of bonding to themselves, or

they can be of two different types, and are capable of bonding to each other. Other possibilities include the use an adhesive between layers and the use of thermoset elastomers.

[120] Given the tremendous diversity of polymer chemistries, precursors, synthetic methods, reaction conditions, and potential additives, there are a huge number of possible elastomer systems that could be used to make microfluidic devices of the present invention. Variations in the materials used depends on the need for particular material properties, i.e. solvent resistance, stiffness, gas permeability, or temperature stability.

[121] There are a variety of types of elastomeric polymers. A brief description of the most common classes of elastomers is presented here, with the intent of showing that even with relatively "standard" polymers, many possibilities for bonding exist. Common elastomeric polymers include polyisoprene, polybutadiene, polychloroprene, polyisobutylene, poly(styrene-butadiene-styrene), the polyurethanes, and silicones.

Polyisoprene, polybutadiene, polychloroprene:

[122] Polyisoprene, polybutadiene, and polychloroprene are all polymerized from diene monomers, and therefore have one double bond per monomer when polymerized. This double bond allows the polymers to be converted to elastomers by vulcanization (essentially, sulfur is used to form crosslinks between the double bonds by heating). This would easily allow homogeneous multilayer soft lithography by incomplete vulcanization of the layers to be bonded; photoresist encapsulation would be possible by a similar mechanism.

Polyisobutylene:

[123] Pure polyisobutylene has no double bonds, but is crosslinked to use as an elastomer by including a small amount (~1%) of isoprene in the polymerization. The isoprene monomers give pendant double bonds on the polyisobutylene backbone, which may then be vulcanized as above.

Poly(styrene-butadiene-styrene):

[124] Poly(styrene-butadiene-styrene) is produced by living anionic polymerization (that is, there is no natural chain-terminating step in the reaction), so "live" polymer ends can exist in the cured polymer. This makes it a natural candidate for the present photoresist encapsulation system (where there will be plenty of unreacted monomer in the liquid layer poured on top of the cured layer).

Incomplete curing would allow homogeneous multilayer soft lithography (A to A bonding). The chemistry also facilitates making one layer with extra butadiene ("A") and coupling agent and the other layer ("B") with a butadiene deficit (for *hetero*geneous multilayer soft lithography). SBS is a "thermoset elastomer", meaning that above a certain temperature it melts and becomes plastic (as opposed to elastic); reducing the temperature yields the elastomer again. Thus, layers can be bonded together by heating.

Polyurethanes:

[125] Polyurethanes are produced from di-isocyanates (A-A) and di-alcohols or di-amines (B-B); since there are a large variety of di-isocyanates and di-alcohols/amines, the number of different types of polyurethanes is huge. The A vs. B nature of the polymers, however, would make them useful for heterogeneous multilayer soft lithography just as RTV 615 is: by using excess A-A in one layer and excess B-B in the other layer.

Silicones:

[126] Silicone polymers probably have the greatest structural variety, and almost certainly have the greatest number of commercially available formulations. The vinyl-to-(Si-H) crosslinking of RTV 615 (which allows both heterogeneous multilayer soft lithography and photoresist encapsulation) has already been discussed, but this is only one of several crosslinking methods used in silicone polymer chemistry.

Cross Linking Agents:

[127] In addition to the use of the simple "pure" polymers discussed above, crosslinking agents can also be added. Some agents (like the monomers bearing pendant double bonds for vulcanization) are suitable for allowing homogeneous (A to A) multilayer soft lithography or photoresist encapsulation; in such an approach the same agent is incorporated into both elastomer layers. Complementary agents (i.e. one monomer bearing a pendant double bond, and another bearing a pendant Si-H group) are suitable for heterogeneous (A to B) multilayer soft lithography. In this approach complementary agents are added to adjacent layers.

Other Materials:

[128] In addition, polymers incorporating materials such as chlorosilanes or methyl-, ethyl-, and phenylsilanes, and polydimethylsiloxane (PDMS) such as Dow Chemical Corp. Sylgard 182, 184 or 186, or aliphatic urethane diacrylates such as (but not limited to) Ebecryl 270 or Irr 245 from UCB Chemical can also be used.

[129] The following is a non-exclusive list of elastomeric materials which can be utilized in connection with the present invention: polyisoprene, polybutadiene, polychloroprene, polyisobutylene, poly(styrene-butadiene-styrene), the polyurethanes, and silicone polymers; or poly(bis(fluoroalkoxy)phosphazene) (PNF, Eypel-F), poly(carborane-siloxanes) (Dexsil), poly(acrylonitrile-butadiene) (nitrile rubber), poly(1-butene), poly(chlorotrifluoroethylene-vinylidene fluoride) copolymers (Kel-F), poly(ethyl vinyl ether), poly(vinylidene fluoride), poly(vinylidene fluoride – hexafluoropropylene) copolymer (Viton), elastomeric compositions *of* polyvinylchloride (PVC), polysulfone, polycarbonate, polymethylmethacrylate (PMMA), and polytertrafluoroethylene (Teflon).

Doping and Dilution:

[130] Elastomers can also be "doped" with uncrosslinkable polymer chains of the same class. For instance RTV 615 may be diluted with GE SF96-50 Silicone Fluid. This serves to reduce the viscosity of the uncured elastomer and reduces the Young's modulus of the cured elastomer. Essentially, the crosslink-capable polymer chains are spread further apart by the addition of "inert" polymer chains, so this is called "dilution". RTV 615 cures at up to 90% dilution, with a dramatic reduction in Young's modulus.

Pre-Treatment and Surface Coating

[131] Once the elastomeric material has been molded or etched into the appropriate shape, it can be pre-treated in order to facilitate operation in connection with a particular application. For example, in sorting biological entities such as cells or DNA the hydrophobic nature of the biological entity can cause it to adhere to the hydrophobic elastomer of the walls of the channel. Therefore, it is useful to pre-treat the elastomeric structure order to impart a hydrophilic character to the channel walls. In an embodiment of the present invention utilizing the General Electric RTV 615 elastomer, this can be accomplished by boiling the shaped elastomer in acid (e.g. 0.01% HCl in water, pH 2.7, at 60 °C for 40 min).

[132] Other types of pre-treatment of elastomer material are also contemplated by the present application. For example, certain layers of elastomer can be pre-treated to create anchors (i.e., immobilization site) for surface chemistry reactions (for example in the formation of peptide chains), or binding sites for antibodies.

[133] Still other types of treatment include coating the microfluidic device, in particular the surface of fluid flow channel, with a non-reactive compounds such as Teflon® and other relatively non-reactive polymers. Such surface treated microfluidic devices are useful in conducting chemical reactions or assays as the fluid flow channels are rendered inert to such processes.

[134] Alternatively the microfabricated microfluidic device can be subjected to a chemical treatment process to remove any leachable materials within the elastomeric polymer block. For example, GTE RTV 615 polymer can be washed or soak in one or more organic solvents to remove any unreacted monomers. By removing such unreacted monomers reduces any potential background noise in the analytical device analysis or possible side-product in chemical reactions. Suitable organic solvent for removing unreacted monomers in the elastomeric materials include aromatic hydrocarbon solvents, such as toluene, xylene, and benzene; nitrile compounds, such as acetonitrile; halogenated solvents, such as dichloromethane and chloroform; and other organic solvents known to one skilled in the art.

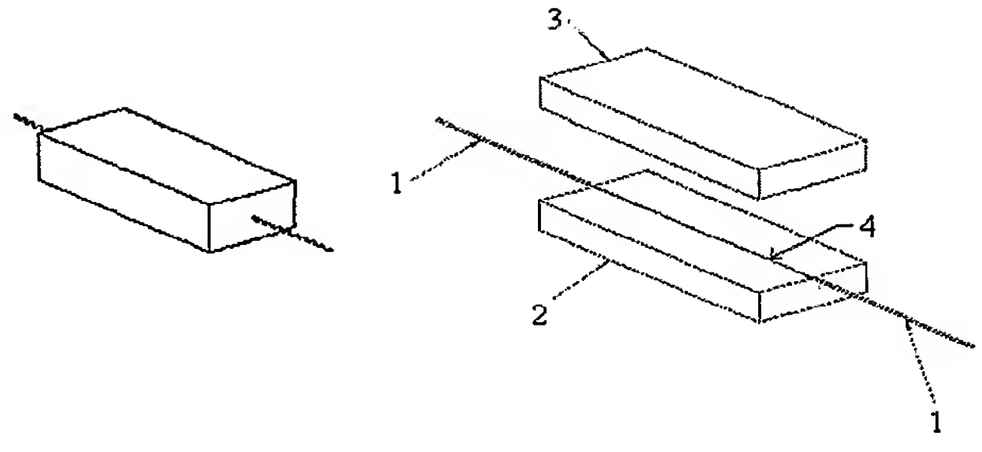
Methods of Operating Microfluidic Devices of the Present Invention

[135] Methods for pumping fluids and opening or closing valve systems are disclosed in the above mentioned U.S. Patent Application Serial No. 09/605,520, which was previously incorporated by reference in its entirety.

[136] Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting.

EXAMPLES

[137] For an initial study, a passive single channel PDMS device design shown below were prepared.



Scheme A

where (1) is a capillary tube, (2) and (3) are a base layer and a cover layer of PDMS block, and (4) is a fluid flow channel.

[138] The fluid flow channel (4) had dimension of 10 μm (height) x 100 μm (width) within a PDMS block, which was sealed to capillary tubes on each end contiguous with the fluid flow channel (4). The PDMS block was designed to accommodate a flow rate of 2-3 μL/min of a variety of solvents. The setup allows easily interface to a conventional ESI mass spectrometry system. Using this configuration, solvent compatibilities and the feasibility of interfacing PDMS chips with LC/MS applications in fields such as pharmaceutical discovery, biochemical analysis, high throughput screening, and proteomics was evaluated.

PDMS Bulk Block Preparation:

[139] Blocks of PDMS were prepared using general procedures disclosed in *Science*, **2000**, *288*, 113-116, and U.S. Patent Application Serial No. 09/09/605,520, filed June 27, 2000. PDMS pre-polymer (GE, lot#KN050) were mixed with curing agent in the ratio of 10:1, 3:1 and 30:1 (mixture weight ~100 grams for each preparations). The pre-mixtures were stir mixed manually using a wood tongue stick. After this, the mixtures were degassed under vacuum for about 40 minutes (until no air bubbles were present in the mixture). After degassing, they were poured on to a bare silicon wafer in aluminum foil covered Petri Dishes. The bare silicon wafer had been silanized with TMCS (trimethylchlorosilane). Samples of each ratio were cured at 80°C in a baking oven. Samples were taken out of the oven at different time intervals in order to observe possible background signal improvement upon extended polymer curing times. The cure times were varied from 3 to 72 hours.

[140] Alignment of a relatively small channel with dimensions (10 μm x 100 μm) to a relative large channel with dimensions 75 μm ID x 370 μm OD in the same z-plane with minimal or zero dead volume is a non-trivial task. The thickness of the capillary wall itself is about 150 μm, which is significantly greater than the PDMS

channel (10 μm x 100 μm). To accommodate a relatively large thickness of the capillary wall, the PDMS base and the cover layer were designed with a sample capillary adapter portion on both sides of the fluid flow channel. In addition, a thick photoresist layer was added on top of the bottom PDMS layer which contains the 10 μm channel on the same master mold.

Mass Spectrometry Test Samples

[141] A strip was cut from each of the bulk PDMS material (5 mm in width and 20 mm in length). Organic solvents such as acetonitrile and methanol were prepared at the desired concentrations with deionized MilliQ-water, and either 0.2 % formic acid or trifluoroacetic acid (mass spectrometry testing buffer). The PDMS testing strips were placed into 2.0 ml sample vials (HP, Teflon coated cap). Testing solvents were then introduced to the vials (1.5 mL solvent in each vial). The PDMS strips were soaked in these solvents for 2.0 hours at room temperature and then removed.

Protein Sample Preparation

[142] To evaluate possible impact from the polymer leachables on mass spectrometry analysis of proteins, protein samples were prepared for mass spectrometry studies.

[143] Angiotensin (II) is a small peptide that consists of three possible positively charged states in acidic solution. A stock solution of Angiotensin (II) was prepared at a concentration of 5.0 mg/mL in water (0.2% TFA). 100 μL of the stock solution were then added to 1.5 mL of the organic solvent that had been treated with the PDMS strips in different component ratios. The resulting solvents were directly infused into ESI-mass spectrometry system for data acquisition.

Mass Spectrometry Studies

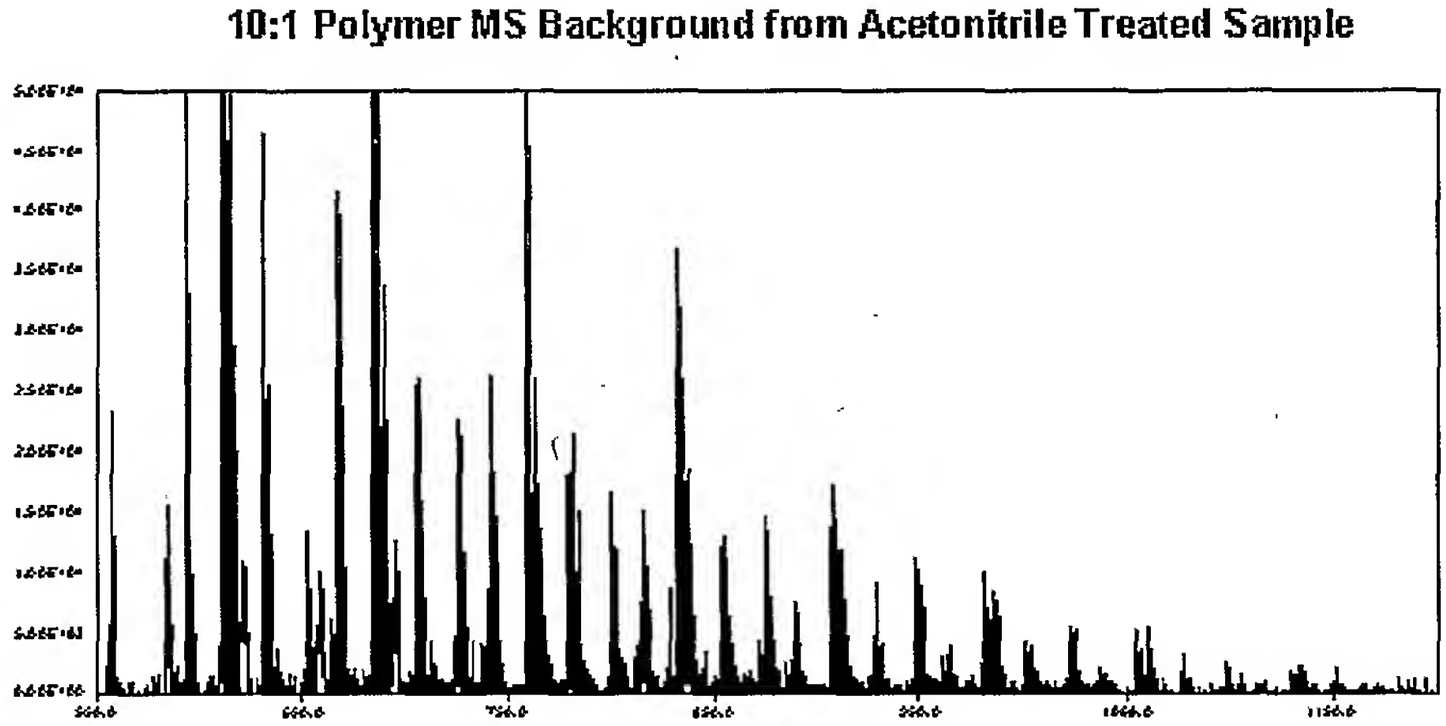
[144] A VG-Platform single quadruple mass spectrometer was used for studies. For bulk material testing, a standard infusion testing method was used. The mass spectrometer condition had been optimized with standard Angiotensin (II) solutions. Samples were introduced to the mass spectrometer from a 250 μL Hamilton syringe through 30 cm long PEEK tubing directly to the ESI source adapter. A Harvard syringe pump was used to drive the liquid stream. All testing in this section was maintained at a flow rate of 10 μL/minute.

[145] Mass spectrometer was programmed to acquire multiple scans (20 scans) in the mass range of 200 amu to 1300 amu, where the most intensive signals due to

polymer background can be observed. Raw data were reduced in two stages by first subtracting the system background and then reducing the spectra to single scan equivalents.

Conclusion and Results

[146] Acetonitrile (i.e., ACN) leaches a complex envelope of low mol. wt siloxanes from the bulk PDMS matrix.



MS-1. PDMS polymer background by ACN in ESI mass spectrometry analysis

[147] MS-1 shown above is a typical mass spectrum of acetonitrile solvent with the leached PDMS polymers. The spectra envelope is centered on a mass-to-charge of about 650 amu and extended to masses as high as 1200 amu. The spectrum consists of repeat units of 74 amu which is believed to correspond to the dimethylsiloxane monomer unit (i.e., $-Si(CH_3)_2-O$).

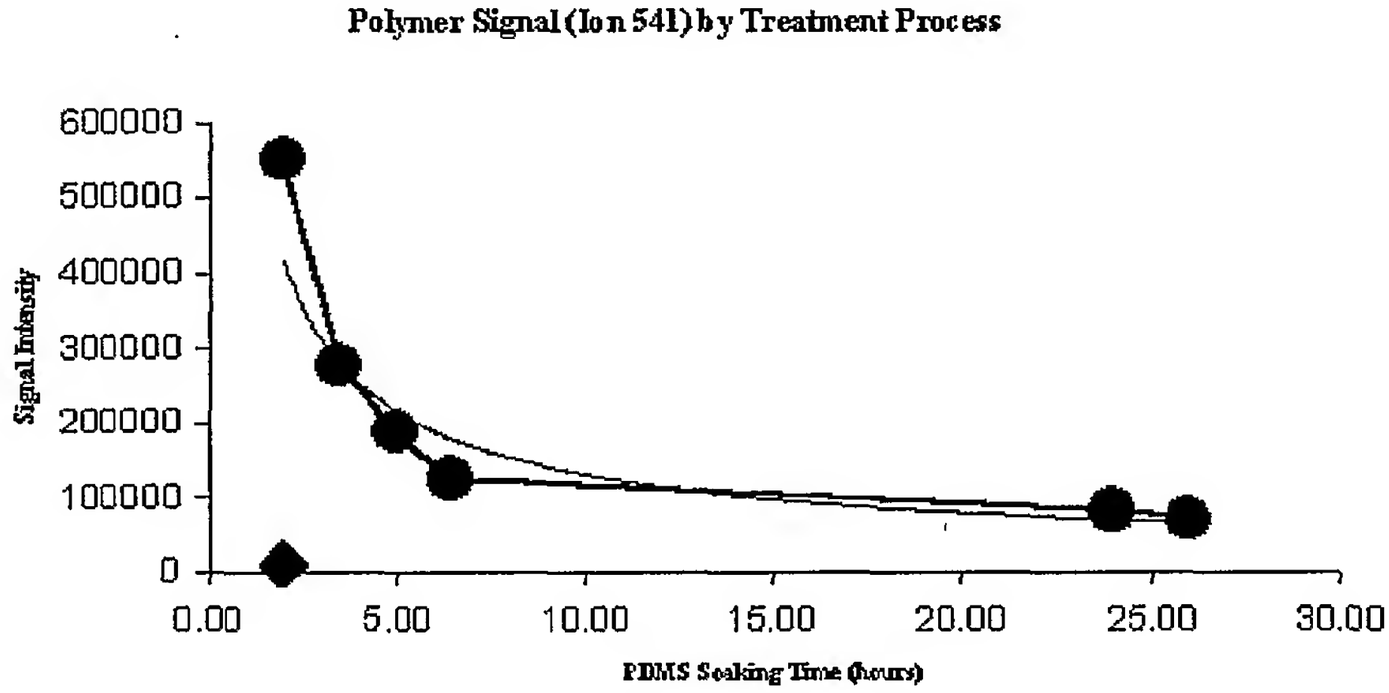
PDMS Surface Cleaning Treatment

[148] As shown in MS-1, polymer leaching in PDMS block based microfluidic device causes background noise in a mass spectrum. To reduce the background noise in PDMS polymer based microfluidic device, a PDMS polymer cleaning process was developed. The process generally involves removing leachable materials by washing or soaking the PDMS polymer with an organic solvent. A wide variety of organic solvents were investigated for PDMS microfluidic device cleaning processes. As discussed in detail below, one particularly useful cleaning solvent is toluene. For comparative purposes, results of cleaning PDMS polymers with acetonitrile and toluene are presented below.

[149] The PDMS polymer block treatment procedure using acetonitrile was as follows: the PDMS polymer block was placed into the first acetonitrile vials and soaked

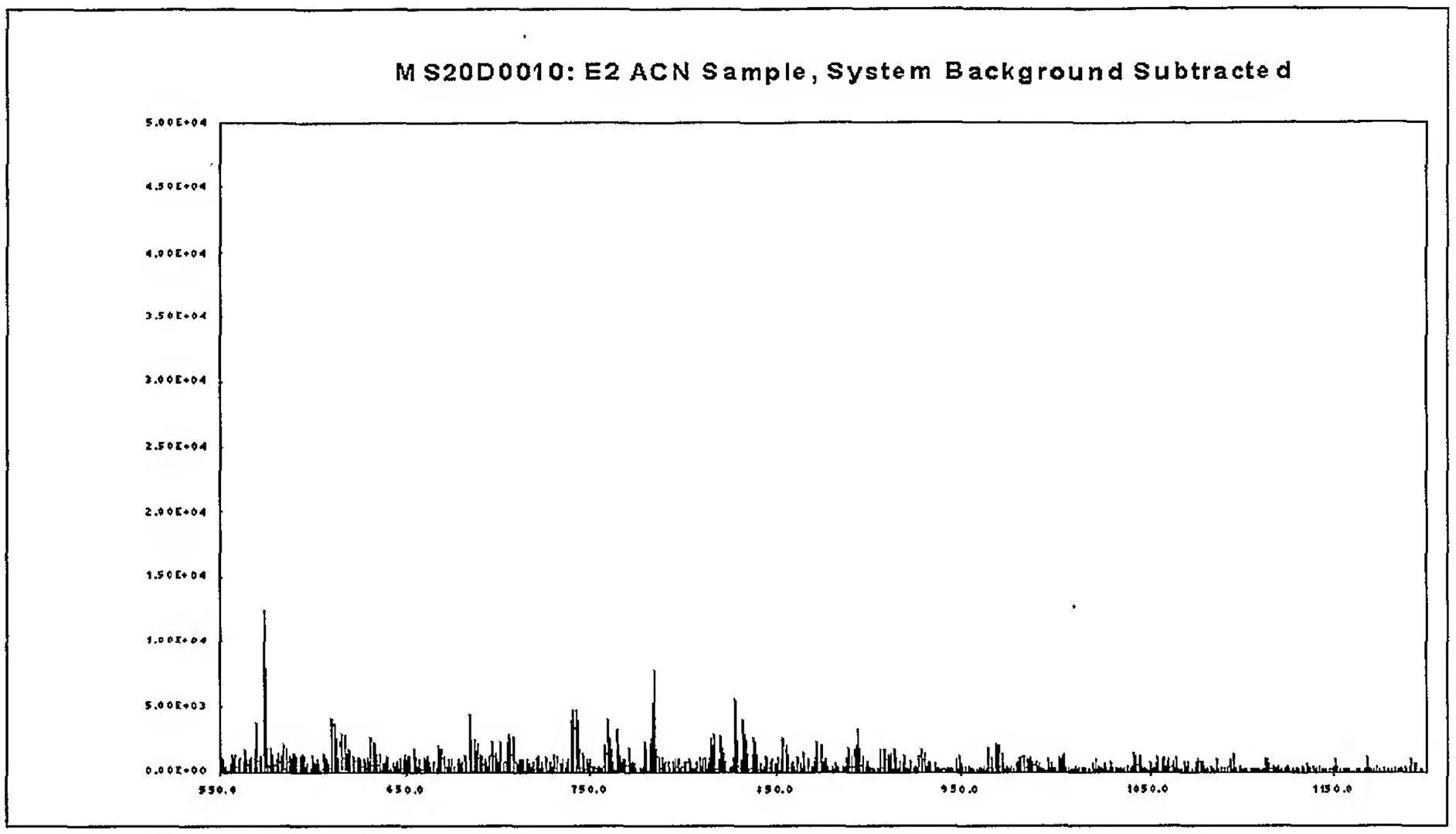
for 2 hours. The PDMS polymer block was transferred to a second acetonitrile solvent and soaked for another 2 hours. This was repeated to obtain a total of 6 acetonitrile washing samples, which were analyzed using a mass spectrometer.

[150] Procedures for cleaning the PDMS polymer block using toluene were similar to the process described above. Except, after the PDMS polymer block was soaked in toluene, the polymer block was transferred to acetonitrile, and this acetonitrile solvent was used as the test solvent because toluene is not compatible with ESI-MS systems.



Graph 1. Polymer Residue Mass Spectrometry Signals by Surface Treatments.

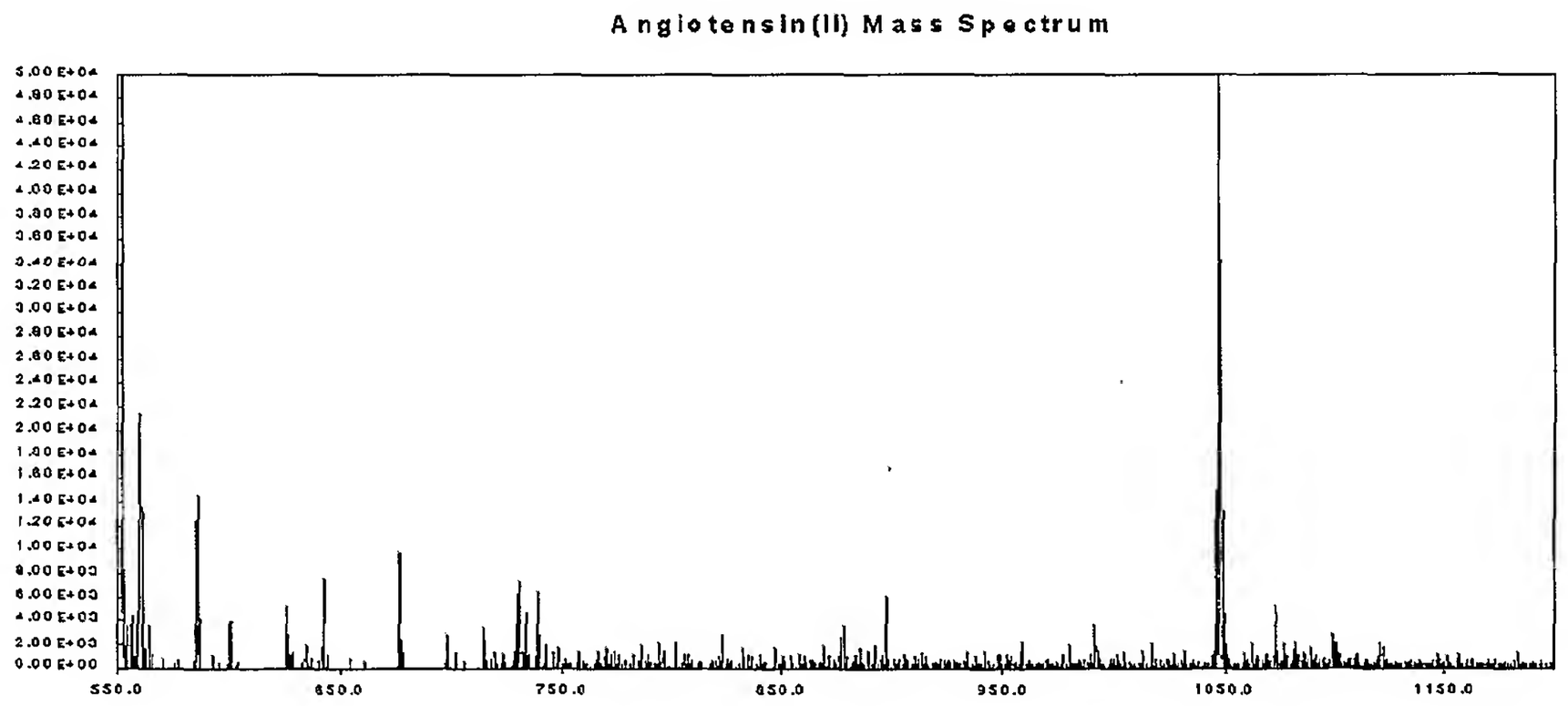
[151] Graph 1 above shows signal intensity of a single polymer ion (m/z 541) after surface treatment with acetonitrile (circle) and toluene (diamond). The results indicate that when acetonitrile was used to wash the polymer block, a typical polymer residue ion (541+) was still detectable (circle) after 24 hours and 6 solvent changes. In contrast, the signal intensity of the same ion was reduced substantially (diamond) after only one washing with toluene. MS-2 shown below is a typical ESI-Mass Spectrum from a toluene washed PDMS polymer block and shows the effectiveness of reducing the background noise by washing the PDMS polymer block with toluene.



MS-2. Mass Spectra of PDMS polymer block after washing with Toluene.

Studies of Signal Loss Impact on Peptide Analysis Caused by PDMS Residues

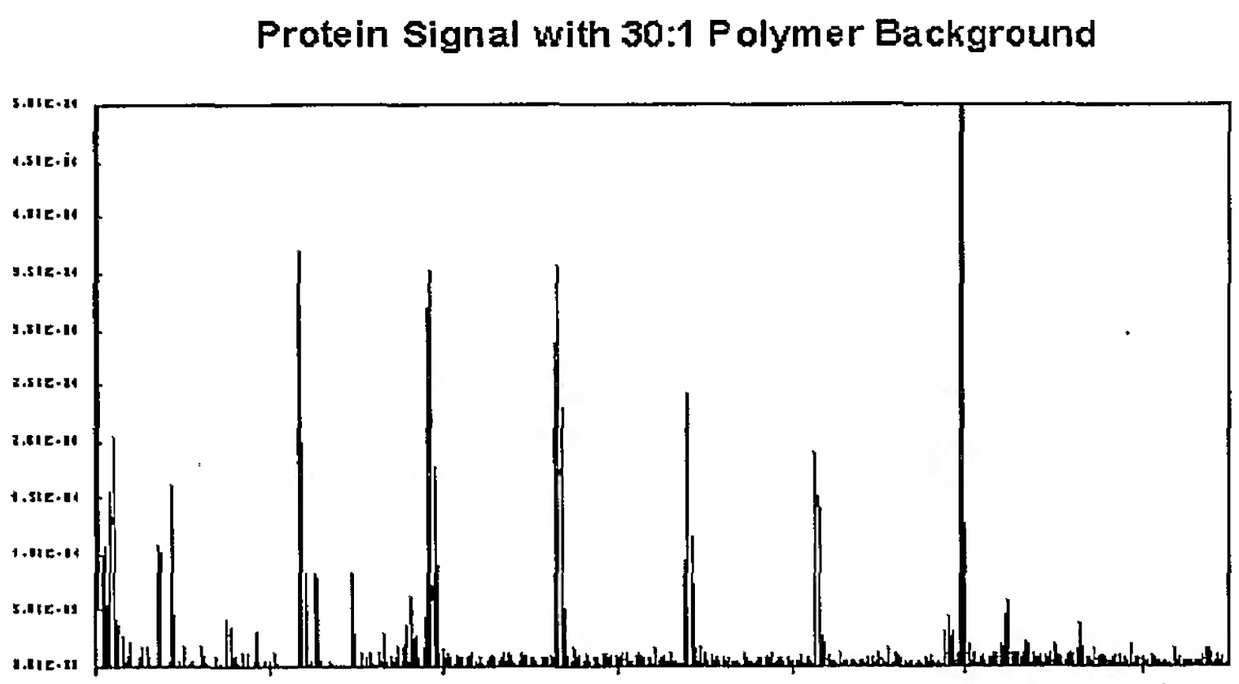
[152] PDMS polymer residues leached into the solvent makes it difficult to interpret mass spectrum. The impact of PDMS polymer leachable materials on a MS analysis was investigated. MS-3 shown below is a typical ESI mass spectrum from Angiotensin (II) which was first dissolved in water and then diluted to 50% with acetonitrile (0.2% formic acid in both solutions).



MS-3. Angiotensin (II) Mass Spectrum in Pure Acetonitrile (50%) Solution

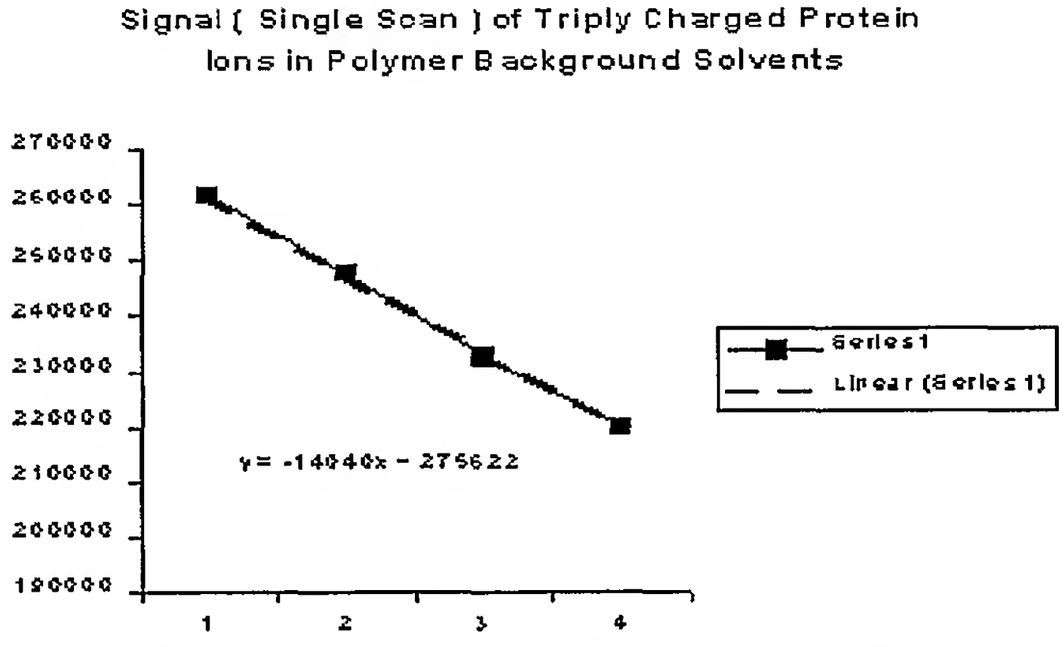
[153] MS-4 below shows a mass spectrum of an angiotensin (II) solution in acetonitrile that was used to soak PDMS (30:1 ratio) polymer block for 2 hours.

Comparision of MS-4 with MS-3 shows the mass spectrum of MS-4 to be significantly more complex, i.e., higher background noise.



MS-4. Angiotensin (II) Mass Spectrum in Acetonitrile Solution which was used to soak a PDMS polymer block.

[154] As MS-4 mass spectrum shows, contaminants (i.e., leachables) from a PDMS polymer block results in a complex background noise and suppresses the signal intensities of the ions derived from the analyte of interest. Depending on the concentration of the analyte and its charge status, as well as other experimental conditions such as flow rate and ionization conditions, signal intensities losses can vary significantly. The amount of signal intensity loss was typically from about 3% to about 20%. Graph 2 below shows the loss of signals upon contaminations produced by different ratios of components A and B of RTV in PDMS preparations.



Graph 2. Angiotensin (II) Signal Loss in polymer-contaminated Acetonitrile (50%) Solution. Treatment#1: 50/50 ACN/Water with 0.2% F.A. Treatment #2: 50/50 ACN (10:1 polymer) /Water with 0.2% F.A. Treatment #3: 50/50 ACN (30:1 polymer) /Water with 0.2% F.A. Treatment #4: 50/50 ACN (3:1 polymer) /Water with 0.2% F.A.

[155] These data show PDMS polymer blocks should be treated to reduce the amount of leachables contaminating the mass spectrum.

PIN structure design for side insert of a large sized tubing to PDMS micro-channels

[156] An interface between the molded microfluidic channel of 10 μm (height) and 100 μm (width), and a capillary tube of 75 μm I.D. and 370 μm O.D in the same z-plane was created using the interface design shown in Figure A below.

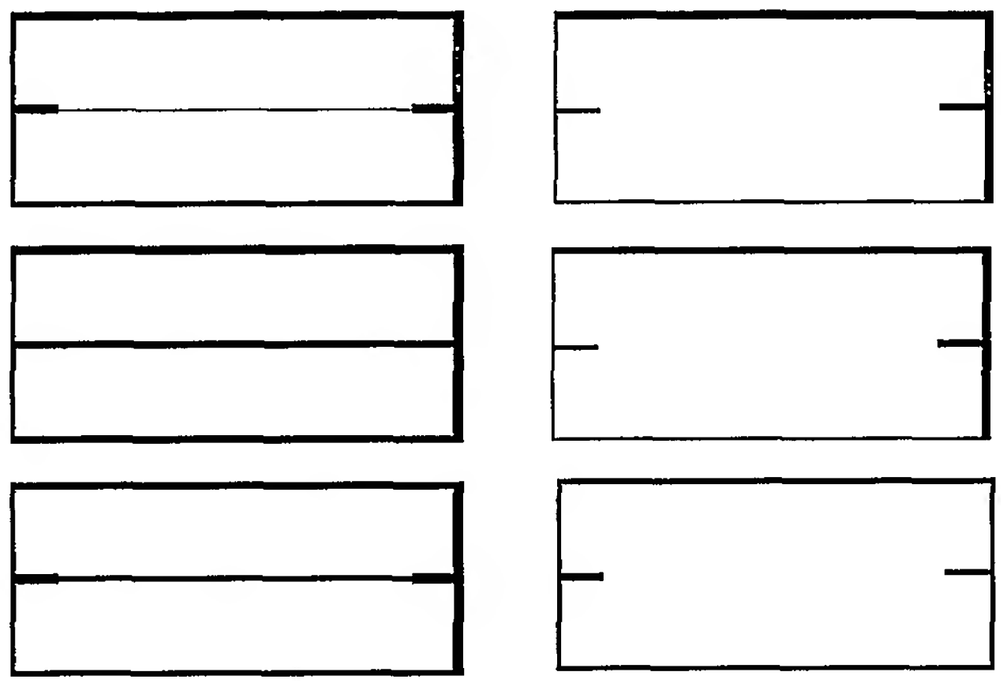


Figure A. Pin port structure mask design.

In Scheme I, the left portion shows masks for patterning of 10 x 100 μm channels and the right portion shows masks to facilitate patterning of large size capillary tube insert port. These masks were used to pattern both top and bottom layers of PDMS polymer block. Upon alignment, the micro-channel and capillary are co-centered with minimal dead volume.

[157] The ports to accept capillary insertion were fabricated using the mask in the right hand panel of Figure A. The height and width of these ports were chosen such that they can accept a capillary having 370 μm OD (as shown in Figure B, right), based on a circumference of about 1150 μm. In this design, the width is fixed at the time of mask design, but the height can be varied.

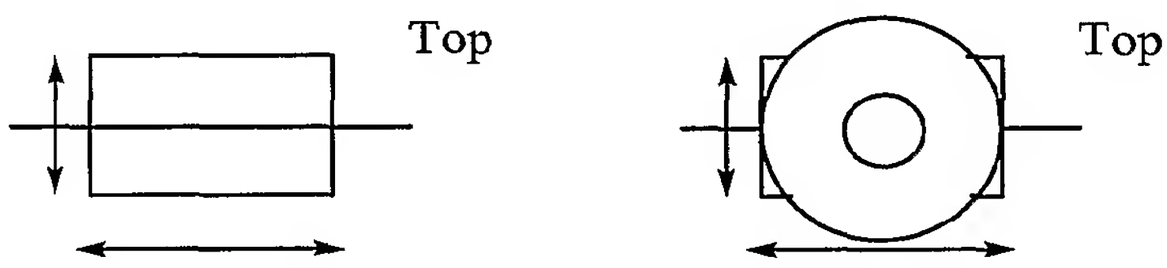


Figure B. Insertion of capillary into port structure.

[158] This design in Figure A is based on the ability to pattern two layers of photoresist. The initial layer consists of the channel itself (Figure A, left). A second, thicker layer of photoresist was then spun onto the pattern and patterned using a second mask (as shown in Figure A, right). The height and width of the raised section created in the second layer creates a capillary adapter portion (i.e., envelope) into which a capillary can be inserted. The contiguity of the two layers of photoresist ensures that the inserted

glass capillary is automatically co-centered with the PDMS micro-channel and the interface between the fluid flow channel and the capillary create a minimum dead volume.

Multiple layer photoresist pattern by soft lithography for a thick second layer

[159] To build the PIN structured master mold on a silicon wafer, a novel multiplayer photoresist coating and developing processes was developed. This process was developed for 4" silicon wafers with channels overlapping each other that vary in size from 100 μm to 300 μm in width (design shown in Figure A). The first layer is 10 μm thick. The thickness of the second layer is about 100 μm.

Multilayer Photoresist Process

A. Process Flow: First (thin) Layer

- Rinse 4" wafer with acetone, isopropyl alcohol (i.e., IPA), water, and IPA thoroughly, use air gun to blow dry wafer. Place cleaned wafer on a clean hot plate setting at 100 °C for 5 minutes.
- Transfer wafer into HMDS bath, equilibrating for 5 minutes.
- Transfer wafer from to photo resist spin coat plate.
- Dispense photo resist 4750 (about 5 mL) on to the center of the silicon wafer slowly.
- Start spin coating at 2000 RPM.
- Soft bake on a hot plate setting at 100 °C for 2 minutes.
- Expose patterning of first layer to UV for 35 seconds.
- Develop the first layer pattern with 4750 developer (1:3 diluted solution).
- After wafer has been dried with nitrogen gas, hard bake the first layer pattern using the following process: start from 100 °C, then setting hot plate to 200 °C. Baking at 200 °C for one hour. Then decrease the temperature down back to 100 °C or lower. Transfer them to a wafer container for further processing.

B. Process Flow: Second (thicker) Layer

- Cleaned the wafer with IPA and water and dry on hot plate for at least 5 minutes at 100 °C.
- Transfer wafer into HDMS bath, equilibrating for 5 minutes.
- Transfer wafer from to photo resist spin coat plate.

- Pour photo resist AZ-PLP100-XT (about 5 mL) on to the center of the silicon wafer slowly.
- Start spin coating using 850 rpm for 30 seconds (for 100 μm thickness coating).
- Soft bake in oven (90 °C for 90 minutes, or alternatively on a hot plate at 100 °C for 5 minutes.
- Transfer to a mask aligner. Align the second photo-mask to the first pattern (from Step A above) on the substrate accurately using the mask aligner. Expose pattern of the second layer to UV for 150 seconds.
- Develop second layer pattern with AZ PLP 100XT developer (1:3 diluted solution).
- After wafer has been dried with nitrogen gas, leave at room temperature overnight (can been kept in the humidity controlled cabinet).
- Hard baking step 1: 90 °C oven 3 hours.
- Hard baking step 2: on a hot plate, starting from 90 °C, increase to 115 °C and maintain at 115 °C for 30 minutes, and then increase to 125 °C for 45 minutes. Reduce the temperature to below 100 °C; and transfer to a container for use.

<u>Sandwich PDMS and face-to-face PDMS block bonding</u>

[160] One can treat the bottom and the cover layers of the PDMS polymer with plasma, e.g., oxygen or water plasma, to introduce an active functional group which can be used to bind the two layers together. Alternatively, the following method of "sandwiching" face-to-face PDMS layers can be used. Such method generally involves:

- Upper pattern and bottom pattern layers were made by spin coating a thin film of RTV premixture on the respective wafer (e.g., 3:1 and 30:1, respectively, at 800 rpm).
- A large amount of 10:1 premixture is prepared separately and poured into two separate blank wafers after vacuum degassing.
- Pattern was cured on the upper and the bottom layer wafers at 80 °C for 30 minutes and 40 minutes, respectively.
- Blank 10:1 RTV substrate pieces were cured at 80 °C for 12 minutes.
- A 10:1 substrate was laid on top of 3:1 or 30:1 patterned layers.
- Substrate and membrane layers were cured together for 15 minutes at 80 °C.
- Both "sandwich" layers were removed (e.g., peeled) from master molding wafers.

- Appropriate layer size was cut and aligned under a microscope to a desired PIN structure.
- The aligned device was baked at 80 °C for 1.5 hours for the face to face bonding.

Capillary tube insertion and device finishing

[161] The tubing walls of both internal and external wall surfaces are coated with a thin layer of polyimide to increase the mechanical strength the capillary. In addition, the polyimide coating reduces chemical interaction between the solvent and the fused silicon capillary. To enhance the bonding of the capillary to the PDMS polymer block, the polyimide coating on the external surface of the capillary was removed.

[162] To enhance the robustness of the inserted capillaries, a casting mold was made to allow a final block molding process. Figure C illustrates the two pieces of this casting mold. It has a slot about 0.5 mm width in both sides of the top portion, which can be cast in clear acrylic. The slot is designed to allow the assembled sealed device with the inserted capillaries to be placed inside and lowered onto the base. The base of the mold, which can be fabricated from Delrin®, is recessed to accommodate the top section and form a tight seal that contains uncured elastomer.

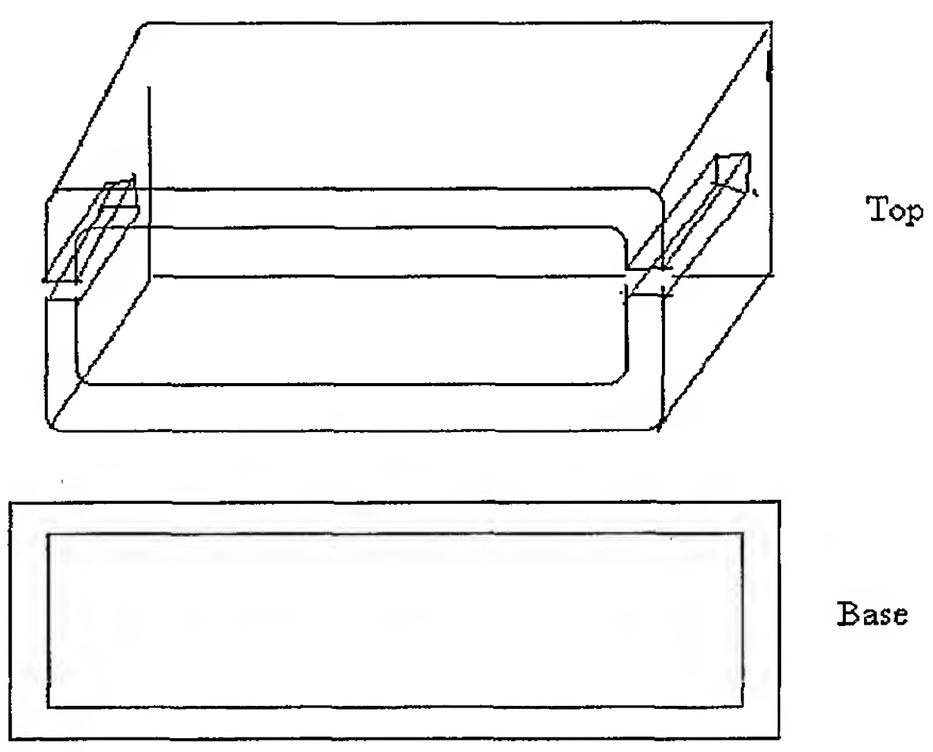


Figure C: Schematic of the mold devices

The PDMS device with the capillary inserted in both sides is placed in the casting mold. The slots on both sides are blocked, e.g., with a several layer of aluminum foil. A degassed 10:1 RTV polymer premixture is poured into the case, and the mold is then placed in an oven for final curing (e.g., 1.5 hours at 80 °C). The aluminum inserts are removed from the slots, and the PDMS polymer microfluidic apparatus together with the upper acrylic case is removed from the Teflon® base. The PDMS polymer microfluidic

apparatus is then removed from the acrylic case. The PDMS polymer microfluidic apparatus can be further treated to reduce the amount of leachable materials.

Chemical treatment of PDMS polymer microfluidic apparatus

[163] The PDMS polymer microfluidic apparatus fabricated as described above was hooked to a conventional solvent delivery pump for washing. A Hamilton syringe and the capillary tube from PDMS polymer microfluidic apparatus was connected directly using Upchurch zero-dead volume unions and ferrules. Tubing sleeves were used on both sides to seal the connections. The pump was started at a flow rate of 5.0 μL/min, and then when the solvent appeared (i.e., exited) at the other side of the device, the flow rate was reduced to 2.0 μL/min. Waste was collected with a capped sample vial bottle. There is a 0.5 mm diameter opening in the cap that allows the fused silica capillary from the device to be inserted directly into the waste collection bottle.

[164] The PDMS polymer microfluidic apparatus were treated with toluene for about 15 hours. About 1.8 mL toluene was collected in the waste bottle. After that, the device was flushed with acetonitrile for 30 minutes.

6.2 Mass Spectrometry Tests

[165] The toluene washed PDMS polymer microfluidic apparatus was set up directly on-line with the ESI ion source using the following procedures:

- The solvent was delivered using a Harvard Syringe pump. A 250 μL Hamilton Syringe was pre-filled with testing solvent. It was connected to a zero-dead volume Upchurch union with finger-tight nuts and ferrules using tubing sleeves.
- The other side of the union was connected to the capillary tube using capillary tubing sleeves.
- Start the solvent delivery first with a flow rate of 5 μL/min. Wait for about 1 to 2 minutes. Solvent can be seen flushing through the PDMS channel and solvent drops then appear at the exit end of the capillary.
- The output capillary can then be connected to a transfer tubing (PEEK tubing or capillary tubing) into the ESI spray head of the mass spectrometer.
- Flow can be resumed at 5 μL/minute. When signal in mass spectrometer screen begins to appear the flow rate is reduced to about 2 μL/minutes.

[166] Mass spectrum of PDMS polymer microfluidic apparatus showed that the PDMS polymer microfluidic apparatus produced an acceptable background noise in a wide variety of solvent mixtures, including 70% methanol / water (with 0.1% formic

acid) and 60% acetonitrile / water (with 0.1% formic acid) solutions. These solvent systems meet most mass spectrometer applications.

[167] The PDMS polymer microfluidic apparatuses of the present invention are useful in a variety of analytical applications, including in liquid chromatography devices and ESI-mass spectrometers.

[168] The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety.

WHAT IS CLAIMED IS:

1. A microfluidic sample injection apparatus for injecting a fluid sample into an analytical device, said microfluidic sample injection apparatus comprising:

(a) a microfluidic device comprising:

(i) an elastomeric block comprising a fluid inlet and a fluid outlet, and

(ii) a fluid flow channel within said elastomeric block in fluid communication with said fluid inlet and said fluid outlet; and

(b) a sample injection capillary in fluid communication with the fluid flow channel for injecting the fluid sample from the microfluidic device into the analytical device, said sample injection capillary comprising:

(i) a distal end being external to said elastomeric block and capable of being placed in alignment with an injection port of the analytical device to allow injection of the fluid sample into the injection port of the analytical device, and

(ii) a proximal end which is inserted into said fluid outlet of the microfluidic device.

2. The microfluidic sample injection apparatus of Claim 1, wherein said fluid flow channel comprises a sample injection capillary adapter layer proximal to said fluid outlet, and wherein said proximal end of said sample injection capillary is inserted into said elastomeric block within said sample injection capillary adapter layer.

3. The microfluidic sample injection apparatus of Claim 2, wherein said sample injection capillary is a fused silica capillary, or _____.

4. The microfluidic sample injection apparatus of Claim 2, wherein said sample injection capillary forms a hermetic seal with said elastomeric block.

5. The microfluidic sample injection apparatus of Claim 4 further comprising a means for generating an ionized mist from the fluid such that the mist is introduced into the injection port of the analytical device for analysis.

6. The microfluidic sample injection apparatus of Claim 5, wherein the analytical device is an electrospray ionization mass spectrometer.

7. The microfluidic sample injection apparatus of Claim 6, wherein said ionized mist generating means comprises a conducting metal which is operatively connected to an electrospray voltage generator, whereby application of electrospray voltage to said conducting metal by said electrospray voltage generator generates the ionized mist from the fluid sample.

8. The microfluidic sample injection apparatus of Claim 7, wherein at least a layer of said conducting metal is inserted into said elastomeric block such that an electrospray tip of said conducting metal is placed within said fluid channel at a position proximal to said fluid outlet.

9. The microfluidic sample injection apparatus of Claim 7, wherein said conducting metal is coated onto said distal end of said sample injection capillary.

10. The microfluidic sample injection apparatus of Claim 9, wherein said distal end of said sample injection capillary is tapered.

11. The microfluidic sample injection apparatus of Claim 5, wherein said elastomeric block further comprises:

a control channel; and

a deflectable elastomeric layer located between said control channel and said fluid flow channel, wherein actuation of said deflectable elastomeric layer affects flow of the fluid sample within said fluid flow channel.

12. The microfluidic sample injection apparatus of Claim 11 further comprising a sample preparation channel in fluid communication with said fluid flow channel, wherein said sample preparation channel is capable of conducting a chemical reaction, chemical assay, chemical separation or other fluid sample preparation processes.

13. The microfluidic sample injection apparatus of Claim 12, wherein said sample preparation channel comprises:

a rotary fluid flow channel which is capable of forming a closed loop system within said elastomeric block by actuation of appropriate deflectable elastomeric layer(s), and

a means for circulating the fluid sample within said rotary fluid flow channel.

14. The microfluidic sample injection apparatus of Claim 13, wherein said mean for circulating the fluid sample within said rotary fluid flow channel comprises:

a plurality of said control channels, and

deflectable elastomeric layers located between said control channels and said rotary fluid flow channel, whereby a sequential actuation of said deflectable elastomeric layers causes the fluid sample within said rotary fluid flow channel to flow.

15. The microfluidic sample injection apparatus of Claim 12, wherein the microfluidic sample injection apparatus comprises a plurality of the sample preparation channels.

16. A method for producing a microfluidic sample injection apparatus of Claim 1, said method comprising the steps of:

(a) providing a first and a second elastomeric layers each having:

(i) a top surface, and

(ii) a bottom surface,

wherein

the top surface of the first elastomeric layer has a microfabricated recess which forms the microfluidic channel and the top surface of the sencod elastomeric layer optionally has a microfabricated recess for integrating the sample injection capillary; and

(b) attaching the top surface of the first elastomeric layer to the top surface of the second elastomeric layer to form a seal between the first and the second elastomeric layers, and integrating the proximal end of the sample injection capillary within the interface of the first and second elastomeric layers proximal to the fluid outlet, or

placing the proximal end of the sample injection capillary within the microfabricated recess of one of the elastomeric layer and attaching the other elastomeric layer to form a seal between the first and the second elastomeric layers.

17. The method of Claim 16, wherein each of the elastomeric layers is produced by a mixture of two monomers.

18. The method of Claim 17, wherein the top surface of the first elastomeric block comprises an excess of one monomer and the top surface of the second elastomeric block comprises an excess of the other monomer relative to the ratio of each monomer needed to form an elastomeric block with substantially no reactive polymerizing functional group.

19. The method of Claim 16 further comprising inserting at least a layer of a conducting metal through the elastomeric block into the fluid flow channel proximal to the fluid outlet such that when an electrospray voltage is applied to the conducting metal an ionized mist is generated from the fluid sample in the fluid flow channel.

20. A method for analyzing a fluid sample using an analytical device comprising:

(a) introducing the fluid sample into a sample injection port of the analytical device using the microfluidic sample injection apparatus of Claim 1; and

(b) analyzing the fluid sample using the analytical device.

21. The method of Claim 20, wherein the elastomeric block of the microfluidic device further comprises:

a plurality of control channels; and

a plurality of deflectable elastomeric layers located between the control channels and the fluid flow channel, whereby a sequential actuation of said deflectable elastomeric layers acts as a peristaltic pump thereby causing the fluid sample within said rotary fluid flow channel to flow.

22. The method of Claim 21, wherein the fluid sample is introduced into the sample injection port of the analytical device by the peristaltic pump action produced by selective actuation of the deflectable elastomeric layers.

23. The method of Claim 22, wherein said analytical device is selected from the group consisting of a UV/VIS spectrometer, fluorescence spectrometer, IR spectrometer, gas chromatographic device, liquid chromatographic device, NMR device, mass spectrometer and a combination thereof.

24. The method of Claim 23, said analytical device is a mass spectrometer.

25. The method of Claim 24, wherein the microfluidic sample injection apparatus further comprises a means for exhaust generating an ionized mist from the fluid sample, whereby the ionized mist is introduced into the sample injection port of the analytical device.

26. The method of Claim 25, wherein said means for generating the ionized mist comprises a conducting metal which is operatively connected to an electrospray voltage generator, whereby the application of electrospray voltage to the conducting metal generates the ionized mist from the fluid sample.

27. The method of Claim 26, wherein at least a layer of the conducting metal is inserted into the elastomeric block such that an electrospray tip of the conducting metal is placed within the fluid channel at a position proximal to the fluid outlet.

28. The method of Claim 26, wherein the conducting metal is coated onto the distal end of the sample injection capillary.

29. The method of Claim 26, wherein the tip of said distal end of capillary is tapered.

30. The method of Claim 21, wherein the elastomeric block of the microfluidic sample injection apparatus further comprises a sample preparation channel which is in fluid communication with the fluid flow channel, wherein the sample preparation channel is capable of conducting a chemical reaction, chemical assay, chemical separation or other fluid sample preparation processes.

31. The method of Claim 30, wherein the sample preparation channel comprises:

a rotary fluid flow channel which is capable of forming a closed loop system within the elastomeric block by actuation of appropriate deflectable elastomeric layer(s), and

a means for circulating the fluid sample within the rotary fluid flow channel.

32. The method of Claim 31, wherein said mean for circulating the fluid within the rotary fluid flow channel comprises:

a plurality of the control channels, and

deflectable elastomeric layers located between the control channels and the rotary fluid flow channel, whereby a sequential actuation of the deflectable elastomeric layers causes the fluid sample within the rotary fluid flow channel to flow.

33. The method of Claim 32, wherein the fluid sample to be analyzed is prepared within the sample preparation channel.

34. The method of Claim 33, wherein the fluid sample to be analyzed is prepared within the sample preparation channel by:

(a) conducting a chemical reaction;

(b) conducting an assay;

(c) degrading a peptide or protein;

(d) conducting a chemical analysis;

(e) extraction of analytes from solvents;

(f) extraction of analytes from bodily fluids;

(g) concentration of sample analytes;

(h) affinity purification of an analyte;

(i) digesting a nucleic acid, carbohydrate, lipid or other molecule or mixture of molecules;

(j) separation;

(k) cell growth (mammalian, bacterial or parasite); or

(l) combinations thereof.

35. The method of Claim 34, wherein the fluid sample to be analyzed is prepared within the sample preparation channel by a combinatorial synthesis such that the fluid sample comprises an array of polymers derived from a monomer.

36. The method of Claim 35, wherein the monomer is selected from the group consisting of a nucleotide, amino acid, carbohydrate, lipid, and other precursor for combinatorial synthesis.

37. The method of Claim 34, wherein the fluid sample to be analyzed is prepared within the sample preparation channel by a receptor or an enzyme binding assay.

38. The method of Claim 34, wherein the fluid sample to be analyzed is prepared within the sample preparation channel by binding a target molecule to an array of oligonucleotides, peptides, proteins, oligosaccharides or small molecules.

39. The method of Claim 34, wherein the fluid sample to be analyzed is prepared within the sample preparation channel by an enzymatic degradation of a protein, peptide, oligonucleotide, carbohydrate, lipid, small molecule or mixtures thereof.

40. The method of Claim 30, wherein the microfluidic sample injection apparatus comprises a plurality of sample preparation channel.

41. The method of Claim 40, wherein each fluid sample from the plurality of sample preparation chamber is independently analyzed by the analytical device.

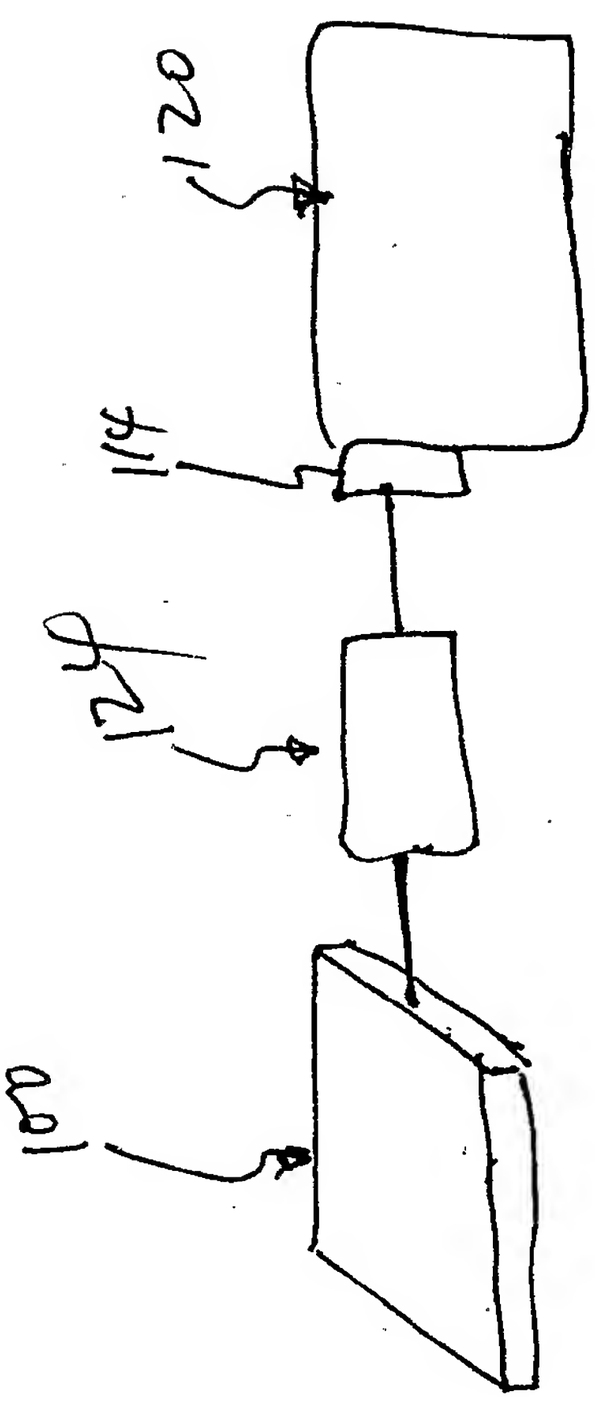


Figure 1A

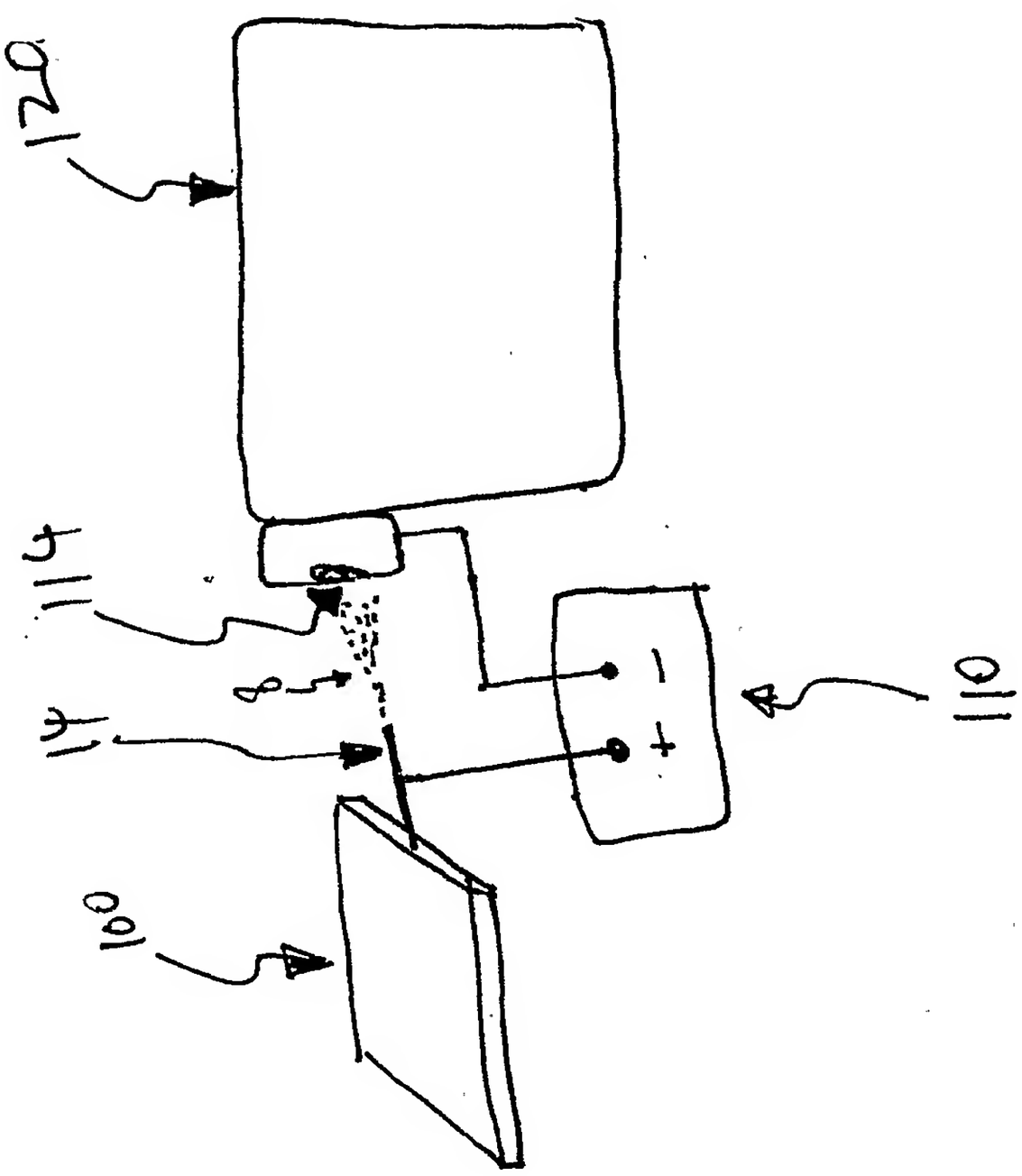


Figure 1B

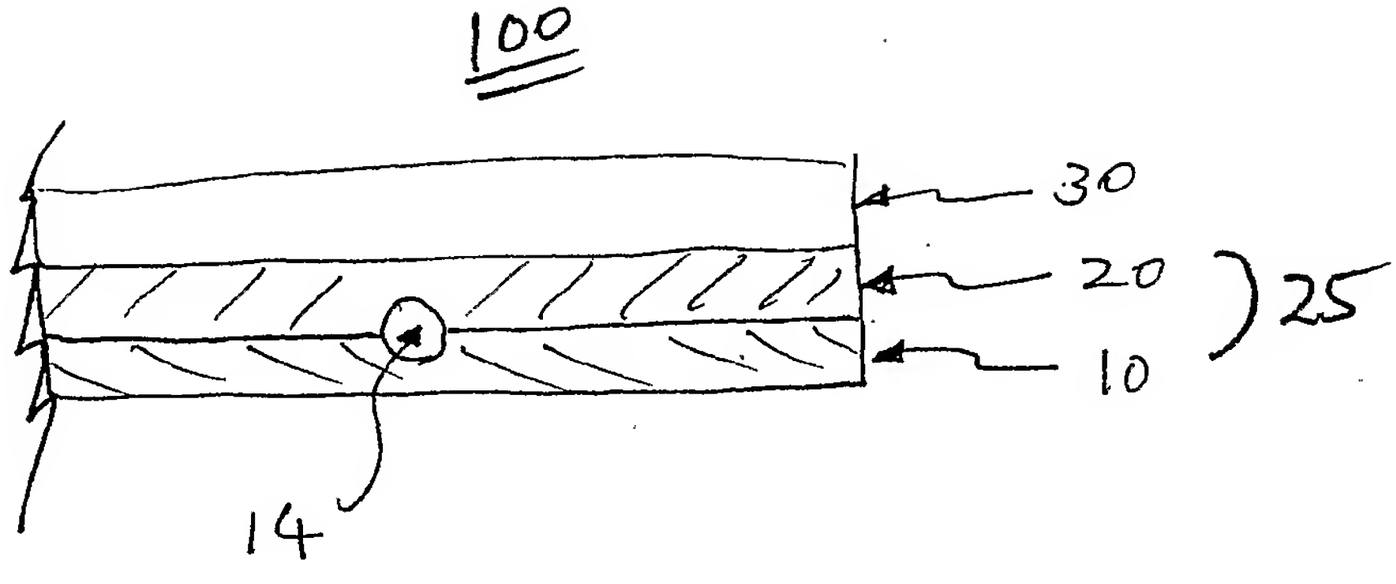


Fig 2A

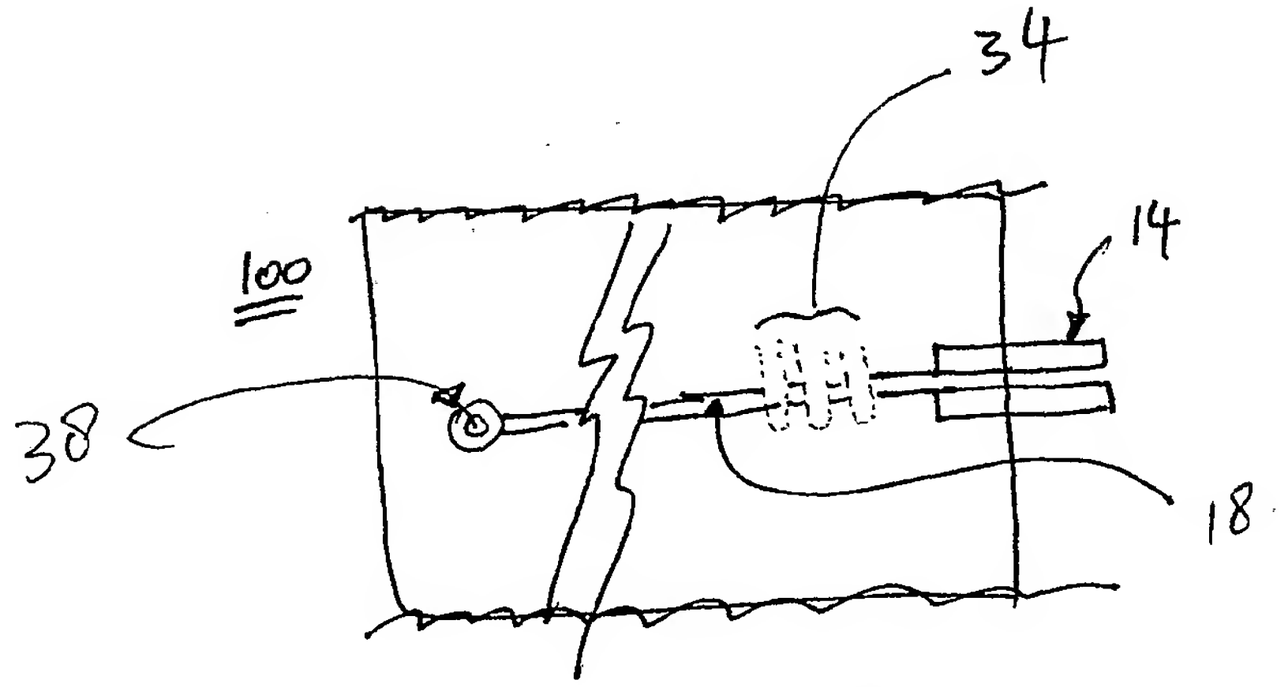


Fig 2B

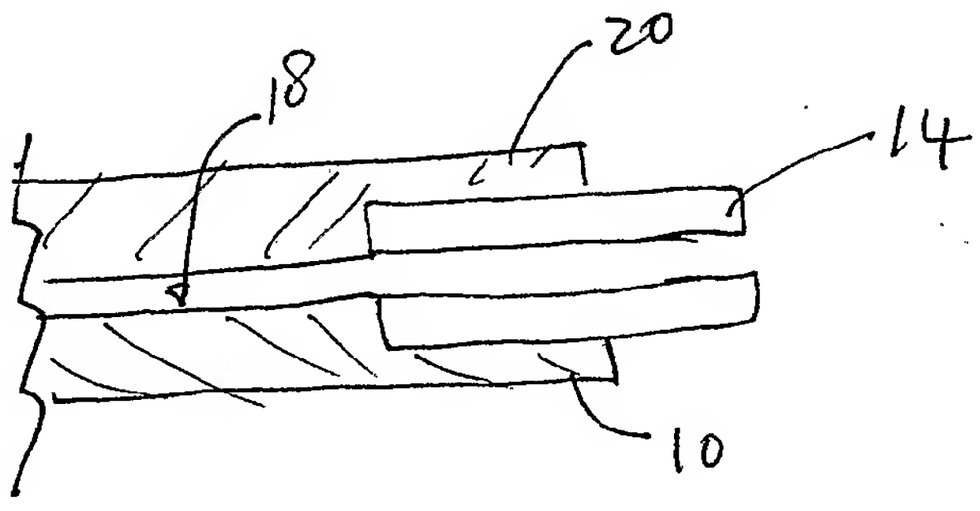


Fig 2C

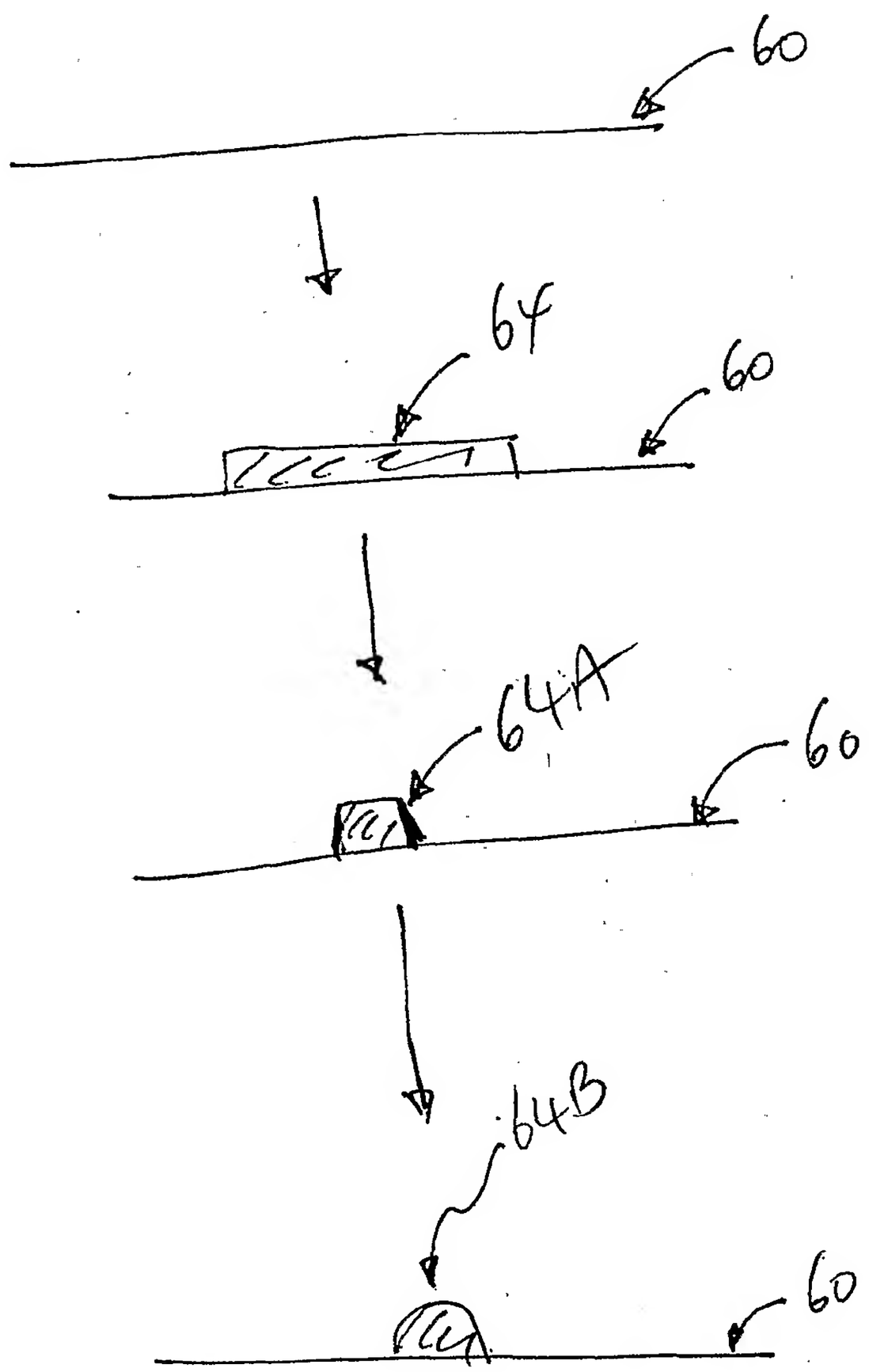


Figure 3A

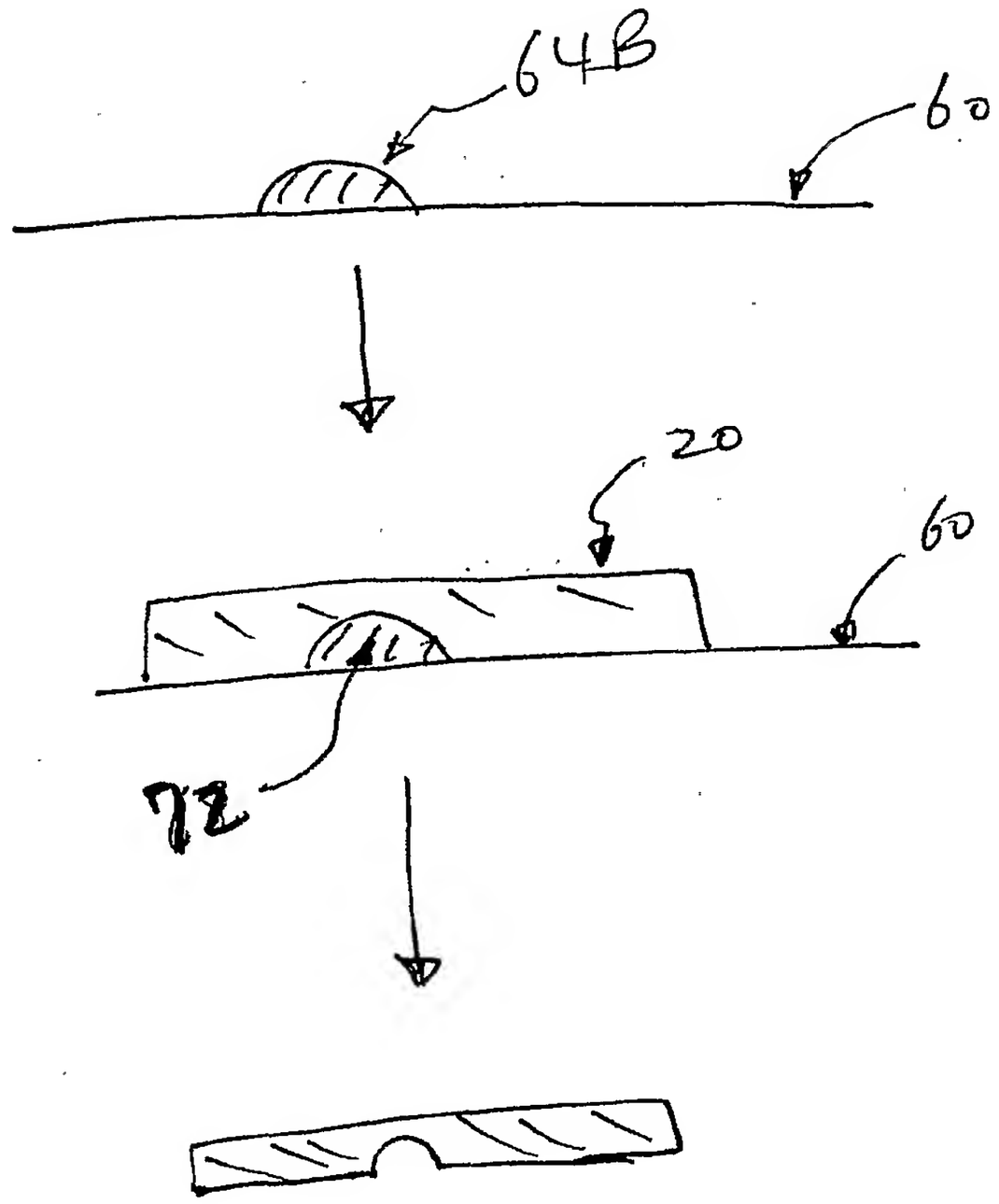
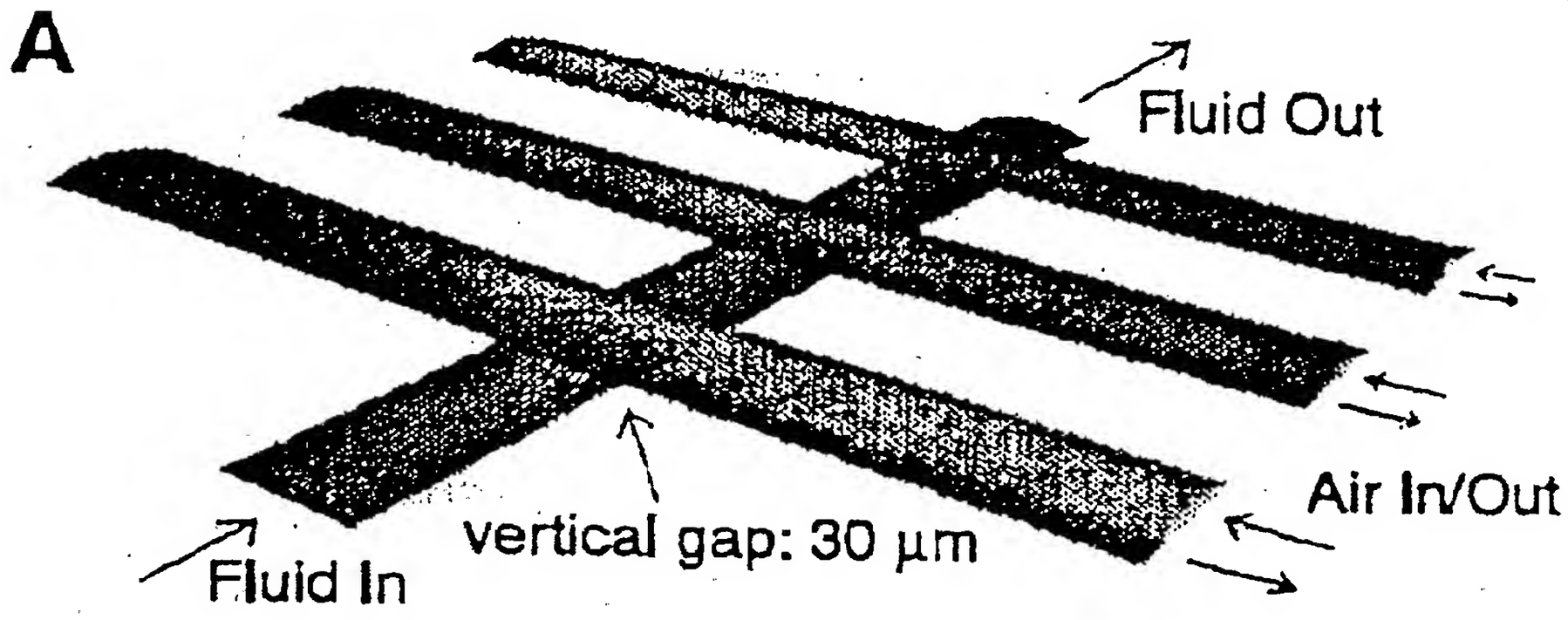


Fig 3B

Fig 4A


A
Fluid Out
Air In/Out
vertical gap: 30 µm
Fluid In

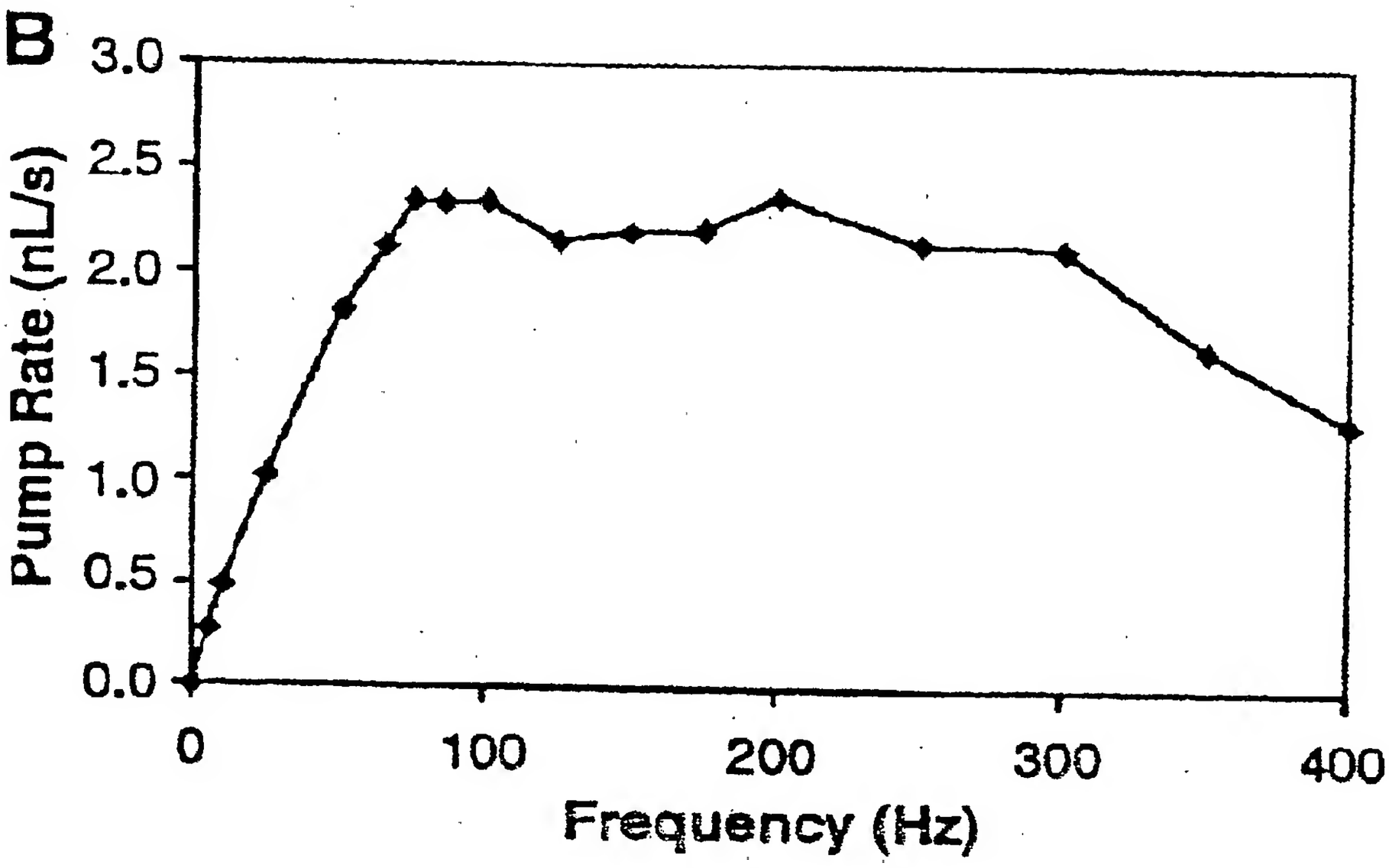

B
Pump Rate (nL/s)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
Frequency (Hz)

Fig 4B

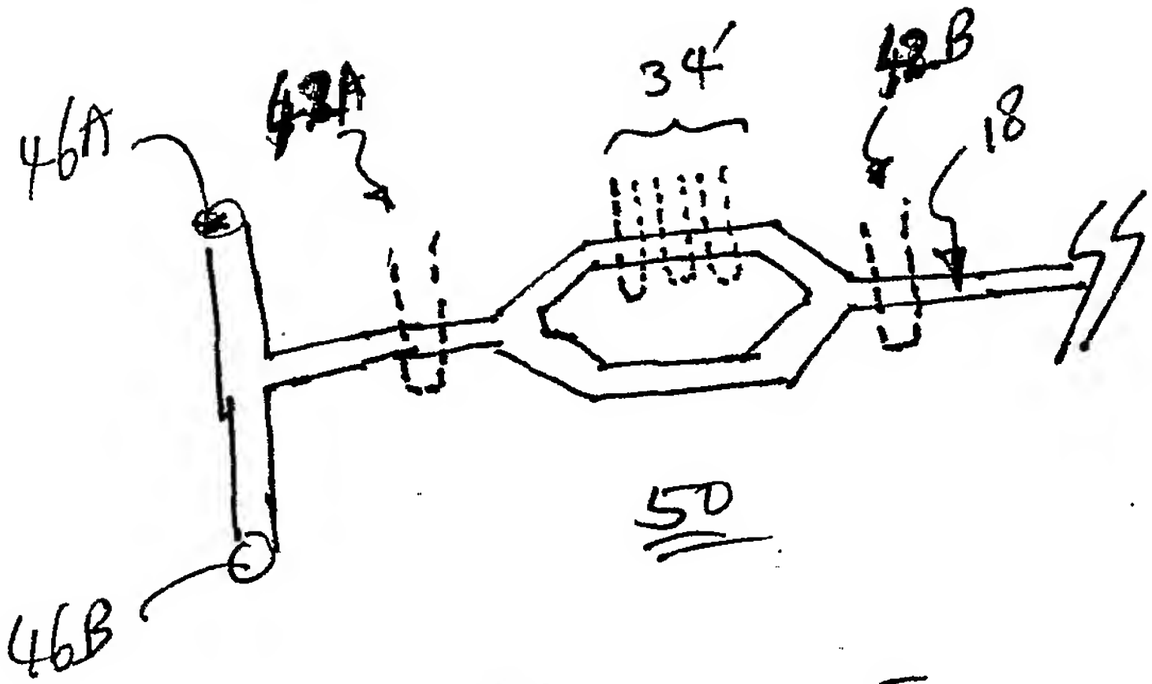


Figure 5

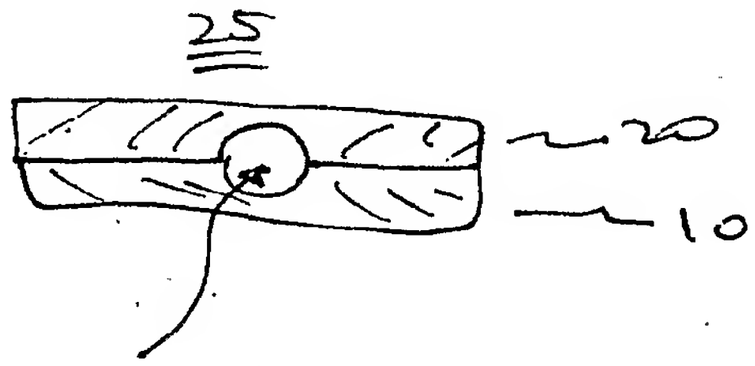


Figure 6A

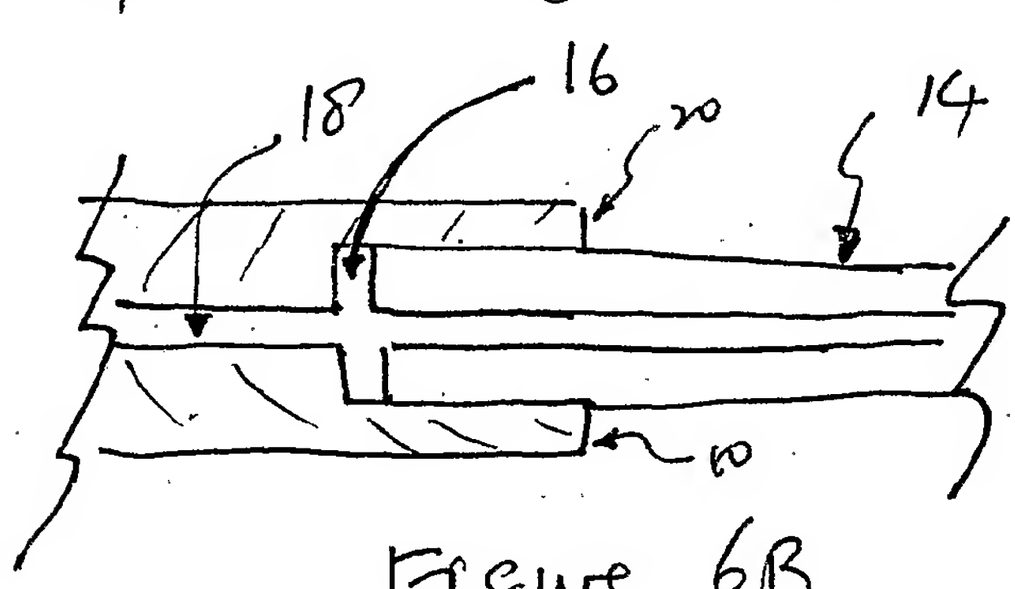


Figure 6B

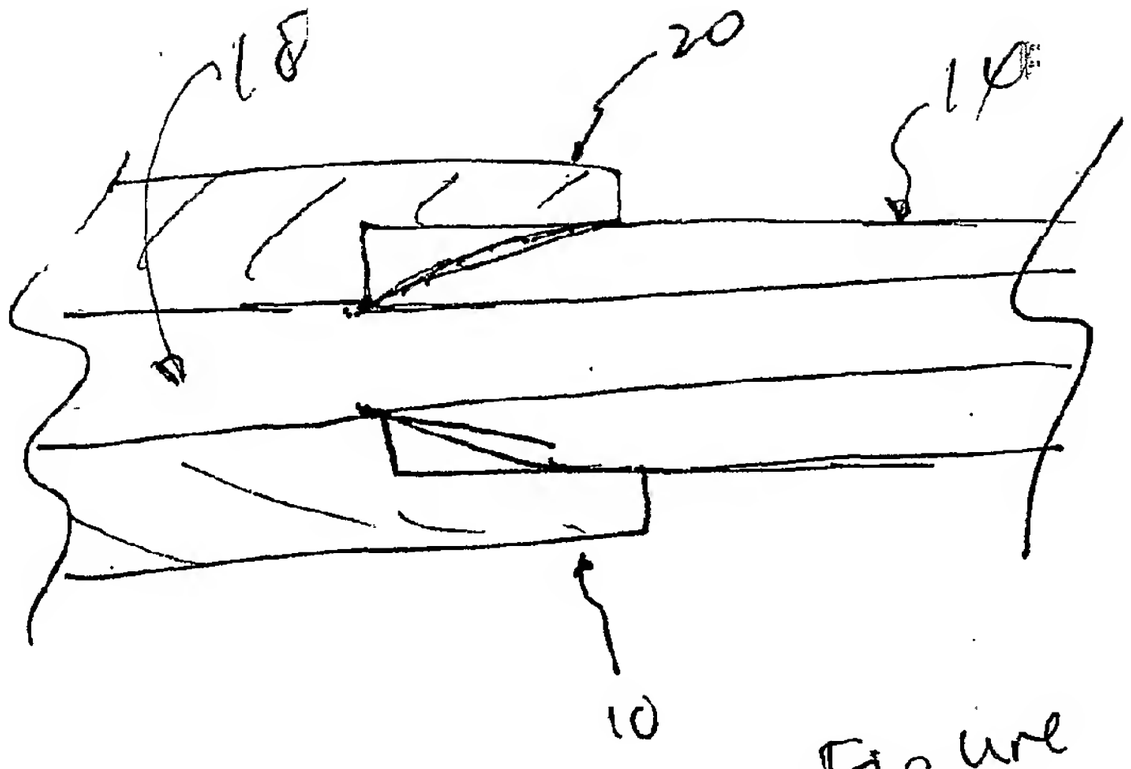


Figure 6C

Figure 7A

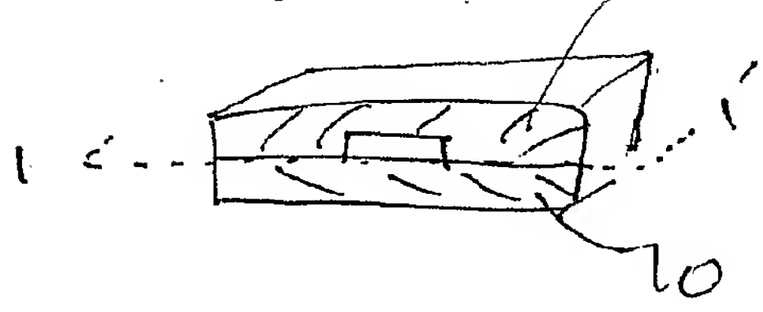


Figure 7B

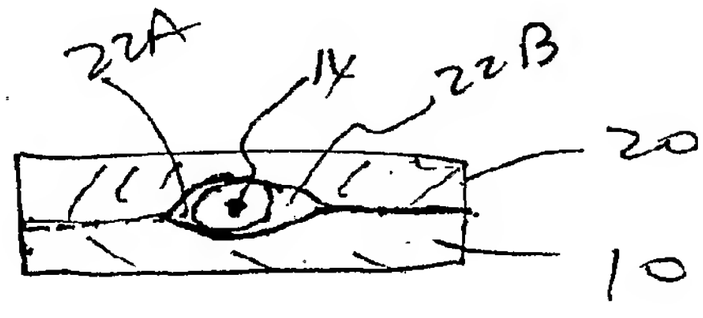


Fig. 7C

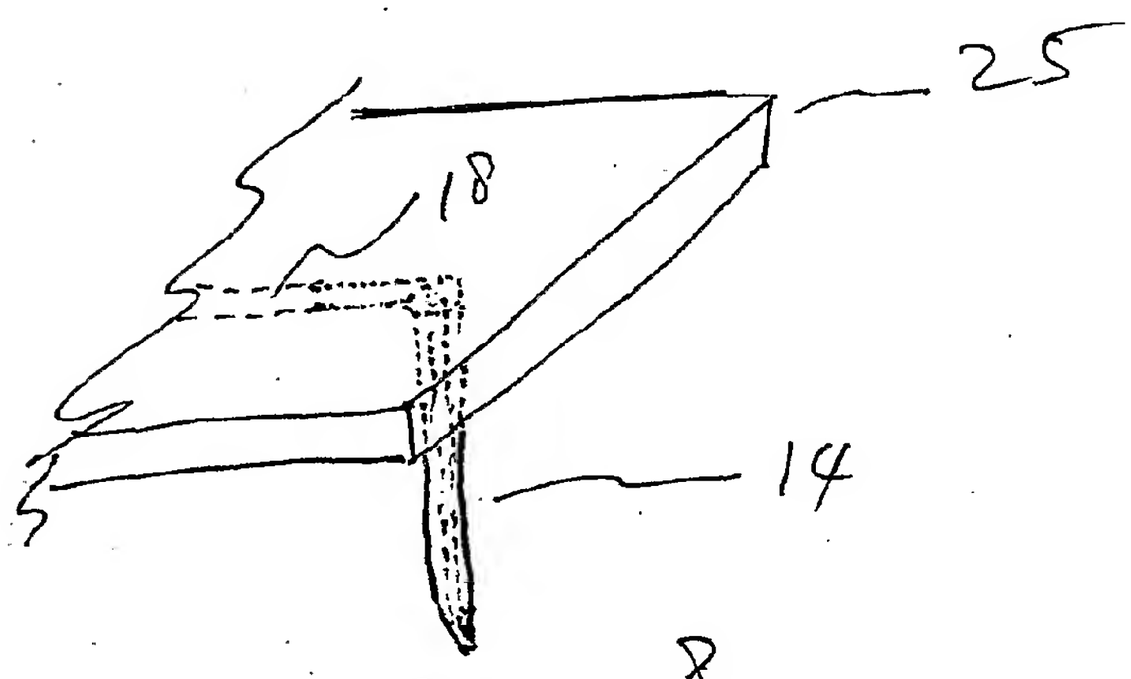


Figure 8

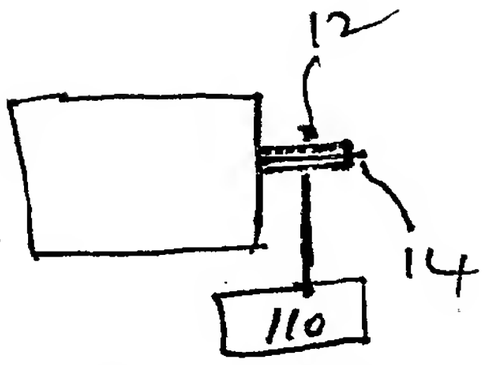


Fig 9A

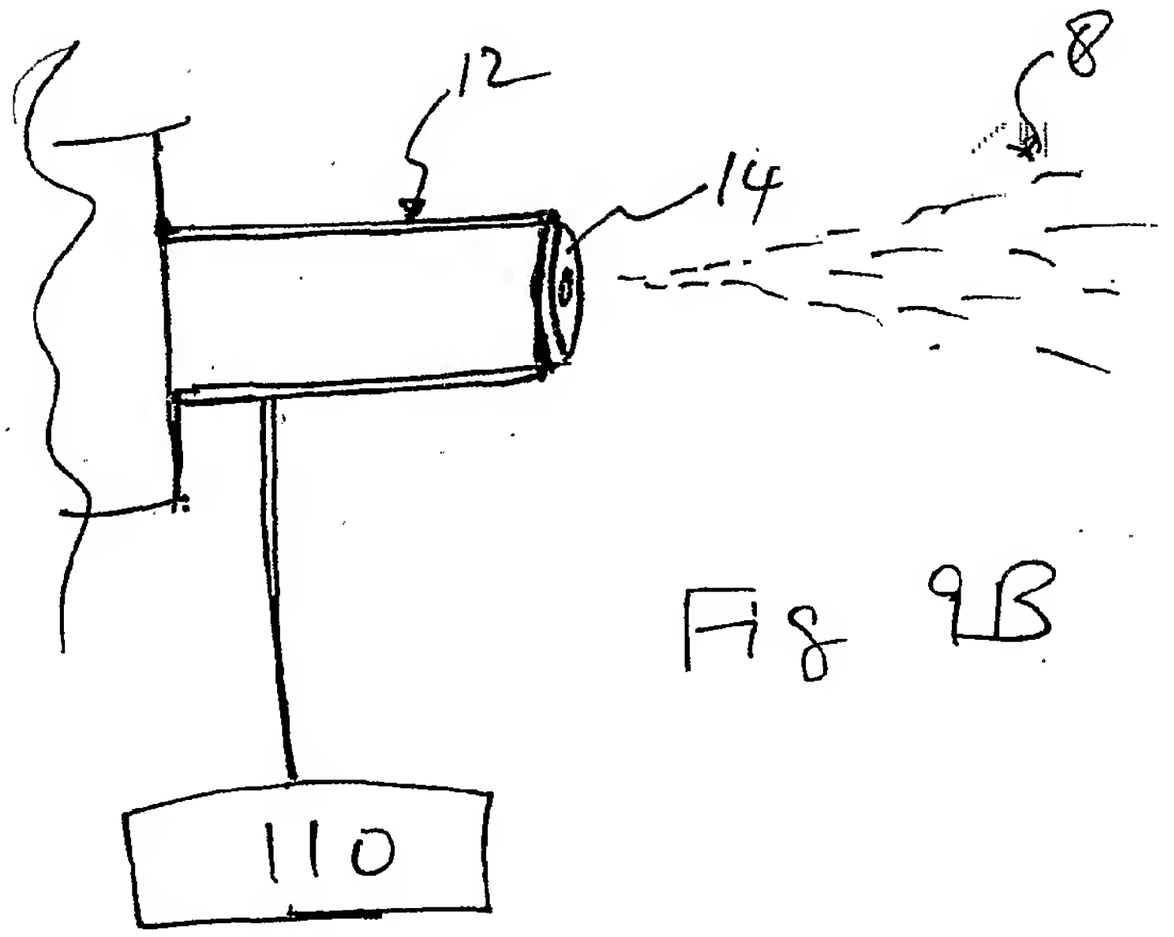


Fig 9B

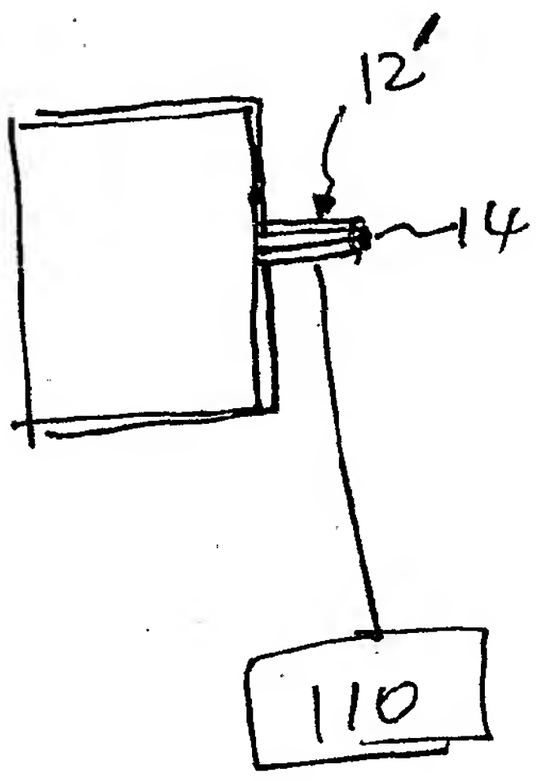


Fig 9C

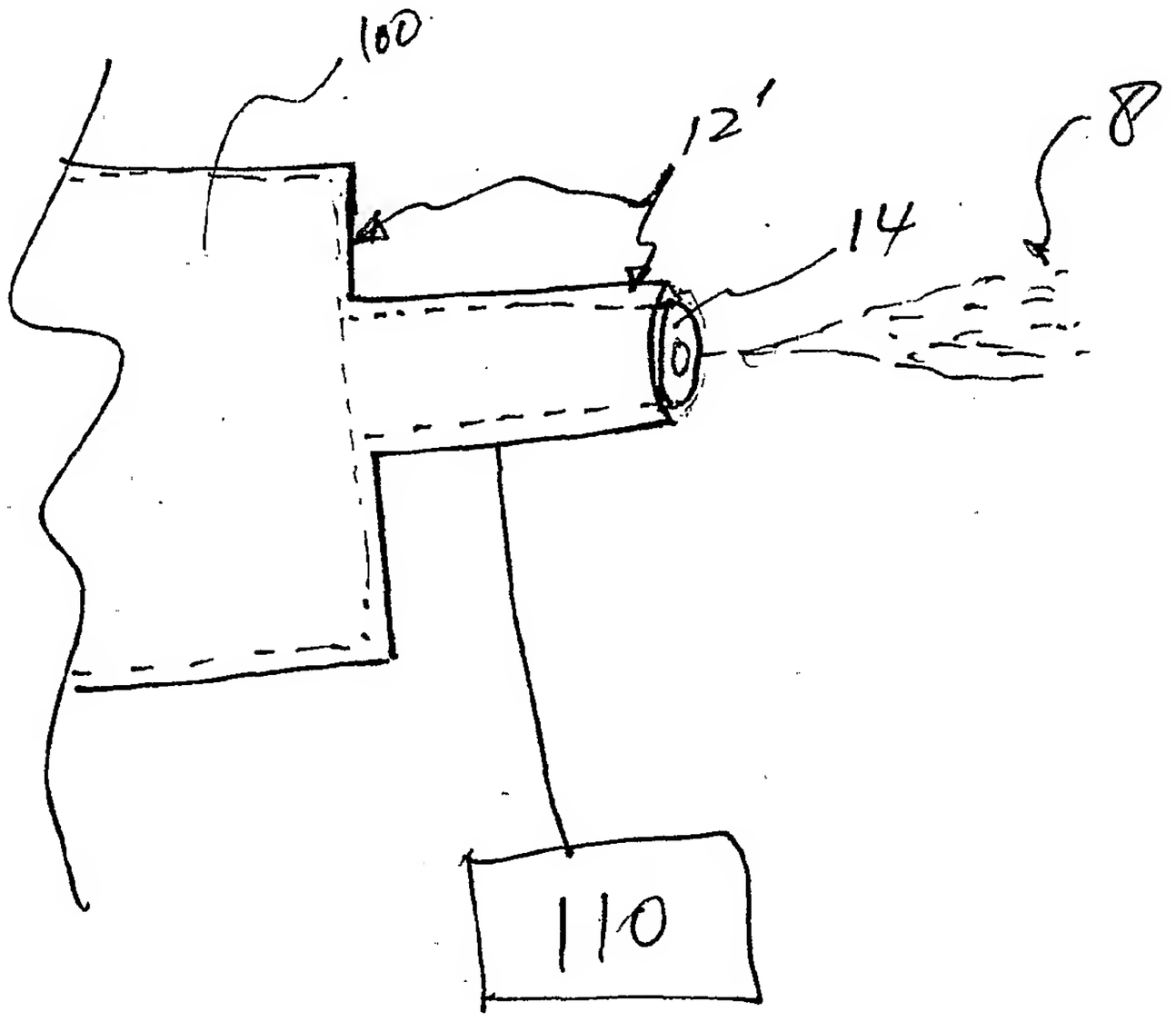


Fig 9D